MAR

# THE JOURNAL
## OF
# GENERAL PHYSIOLOGY

EDITORS
JACQUES LOEB W. J. V. OSTERHOUT

VOLUME V, No. 4 MARCH 20, 1923

PUBLISHED BIMONTHLY
AT MOUNT ROYAL AND GUILFORD AVENUES, BALTIMORE, MD.
BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

COPYRIGHT, 1923, BY THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

Entered as second-class matter November 25, 1918, at the Post Office at Baltimore, Md., under the Act of March 3, 1879.

*Made in the United States of America*

# PUBLICATIONS OF THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

## THE JOURNAL OF EXPERIMENTAL MEDICINE

EDITED BY

SIMON FLEXNER, M.D. PEYTON ROUS, M.D.

*The Journal of Experimental Medicine* is designed to cover the field of experimental medicine. It is issued monthly, two volumes of over 600 pages each appearing in a year. The subscription price per year (two volumes) is $5.00, payable in advance. Single copies cost 75 cents. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of Experimental Medicine*, Mount Royal and Guilford Avenues, Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

## THE JOURNAL OF BIOLOGICAL CHEMISTRY

EDITED BY

STANLEY R. BENEDICT, New York, N. Y. LAFAYETTE B. MENDEL, New Haven, Conn.
H. D. DAKIN, Scarborough, N. Y. DONALD D. VAN SLYKE, New York, N. Y.

*The Journal of Biological Chemistry* is designed for the prompt publication of original investigations of a chemical nature in the biological sciences. It is issued monthly. Each volume consists of as many numbers as are required to make a total of approximately 800 pages. The subscription price is $5.00 per volume to subscribers in the United States and Canada, payable in advance; $5.25 to those in other countries. The number of volumes issued yearly averages four. The price of single copies varies; it is given on the back cover of each number. Remittances should be made by draft or check on New York, or by postal money order, payable to *The Journal of Biological Chemistry*, Mount Royal and Guilford Aves., Baltimore, Md., or Avenue A and 66th Street, New York, N. Y.

## THE JOURNAL OF GENERAL PHYSIOLOGY

EDITED BY

JACQUES LOEB, New York, N. Y. W. J. V. OSTERHOUT, Cambridge, Mass.

*The Journal of General Physiology* is devoted to the explanation of life phenomena on the basis of the physical and chemical constitution of living matter. Information regarding contributions and subscriptions is given in full on the back cover.

## STUDIES FROM THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH

The published results of investigations conducted in the Institute, or under its grants, are republished at irregular intervals in volumes designated *Studies from The Rockefeller Institute for Medical Research*. The subscription price is $2.00 per volume, payable in advance. A special rate of $1.00 per volume is made to those who subscribe to *The Journal of Experimental Medicine*, *The Journal of Biological Chemistry*, or *The Journal of General Physiology*. Remittances should be made by draft or check on New York, or by postal money order, payable to The Rockefeller Institute for Medical Research, Avenue A and 66th Street, New York, N. Y.

## SEMIANNUAL LIST

The Institute publishes a *Semiannual List* of all papers by members of the staff and those working under grants from the Institute, stating the title and place of publication of these reports. The *Semiannual List* will be sent regularly upon application.

# CIRCUS MOVEMENTS OF LIMULUS AND THE TROPISM THEORY.

By WILLIAM H. COLE.

*(From the Laboratory of Biology, Lake Forest College, Lake Forest.)*

(Received for publication, December 13, 1922.)

As a result of asymmetric photic stimulation of many animals locomotion follows a circular path. If the animals are positively phototropic the path bends predominantly towards the stimulated side; if they are negative, towards the non-stimulated side. In such cases crossed innervation from the photoreceptors to the muscles of the locomotor organs on the opposite side must exist, which causes the tonus of the muscles on the two sides to be different (Garrey, 1918–19). For an analysis of animal orientation to light, circus move-

CORRECTION.

On page 228 of Volume V the figures for Ca in *Valonia* sap should be divided by ten. The figures for organic matter should be placed in Column 3 under *Valonia* sap.

lyzed (see Minnich, 1919, for a review of the literature), it has been found that the diameter of the circle turned varies inversely with the light intensity. This is what would be predicted from the tropism theory of Loeb, formulated in 1888, and is contrary to Mast's (1922) most recent theory that orientation is regulated by localized stimulation. A quantitative study of the positive circus movements of *Limulus* herewith presented shows that the photic orientation of this animal accords with the tropism theory, adding therefore one more link in the chain of evidence.

In the experiments[1] adult *Limuli* (from 20 to 60 mm. in diameter) were used, since it was found impractical to use the larval forms. Having determined that the normal animals are positively phototropic

[1] The experiments were performed at the Marine Biological Laboratory, Woods Hole, Mass., and I wish to express my appreciation for the courtesies shown me by the Director and others in charge of the laboratory.

under laboratory conditions, and that the eyes are the important receptors for photic stimulation, the next step was to test animals with a single functional eye. It should be noted that young *Limuli* possess four compound eyes, two large lateral eyes on opposite sides of the cephalothorax, and two small median, anterior eyes, one on either side of the median line. For testing circus movements, it was deemed best to remove or cover both median eyes and one of the lateral eyes, leaving only the opposite lateral eye functional. The removal was accomplished by a fine, sharp scalpel, and the covering by the use of asphaltum. Both kinds of asymmetric animals were allowed recovery periods varying from 4 hours to 2 days, according to whether the eyes were covered or removed. They were then subjected to the photic stimulation of three intensities. A cylindrical glass dish (inside diameter 295 mm.) lined on the inside with tissue paper, and surrounded on the outside by five 40 watt Mazda lamps, contained the single experimental animal. Underneath the dish was a circular cardboard marked off with 4 concentric circles and 4 diameters to facilitate the tracing of the animal's path. The intensity of the light at the center of the dish was not accurately determined, but was approximately 8,000 candle meters. When the lamps were moved outward so that they were 300 mm. from the center the intensity was approximately 2,000 candle meters, and when 450 mm. distant, approximately 900 candle meters. With this arrangement the intensity inside the dish was very nearly constant in all parts, and the illumination was diffuse and non-directive, the only conditions under which circus movements should be tested. The temperature of the water was 24° ± 3°C. Individual trials, usually of 3 minutes duration, were recorded separately on charts like the cardboard described above, except one-fourth smaller. After some practice it becomes easy to trace the path of an animal on the small chart as locomotion occurs in the dish. After the trial with the lowest intensity a test with the highest intensity was repeated to determine whether or not the animal was regular in its reaction. The paths of the animals were measured with a map measurer, and the number of degrees turned per centimeter was calculated, as will be described later. 135 trials on 38 different animals completed the observations.

Before proceeding to a consideration of the results obtained it is necessary to call attention to the fact that *Limuli* freshly collected show a high percentage (about 25 per cent) of irregular reactions to photic stimulation; *i.e.*, some animals will be indifferent to light, others will show a mixture of positive and negative movements, and still others will be definitely positive for a time, and then become the opposite. Among the reasons for such irregularity are the following: (1) the ease with which some individuals are "frightened" by handling; (2) the state of nutrition, "hungry" animals showing rapid movements in many directions; and (3) unknown causes due to previous stimuli. It becomes clear after observing *Limuli*, however, that they are fundamentally and primitively positive to light, but that many factors may modify or mask the phototropic reaction. Such behavior illustrates very well the fact that a primitive reaction of an animal, such as that to light, may be profoundly modified or even obliterated by other reactions occurring simultaneously. The idea of an inclined plane of behavior may be extended to the whole series of animals. Between the lowest forms, whose reactions to light and other stimuli are machine-like in character, and man, whose reactions to the same stimuli are nearly always modified or entirely suppressed by reason, there are all gradations of tropistic behavior. It is not surprising, therefore, to find that *Limulus*, the anatomy of whose nervous system has led to the belief that the animal corresponds closely with the hypothetical ancestors of the vertebrates, shows a modified response many times in respect to light. That this is actually the case is shown by the following experiment which was repeated several times. A normal individual was tested and found to be positive to light; then it was taken up in the hands, turned over, and handled in various gentle ways, and tested again. For several hours afterward this animal was distinctly negative to light, creeping away into the dark as fast as possible, and showing strong stereotropism. On the next morning, however, it showed an equally distinct positive reaction. The effects of handling or "frightening" the animal set up other reactions, including a stereotropic one, which reversed the phototropic reaction. As soon as those effects had disappeared, the primitive positive response to light became again apparent. Before testing animals for circus movements the normal reaction

was always investigated, and animals were selected which showed regularly positive phototropism. Another irregularity which sometimes appeared resulted from suddenly placing an animal under the greatest intensity after having been for some hours in the dim light of the stock aquarium. In these cases rapid movements in many directions occurred. If the intensity was decreased slightly for a few minutes, and then gradually returned to the former amount, regular circus movements appeared. It is concluded from this that in cases where an animal is suddenly subjected to strong illumination the positive phototropism may be obliterated by photokinesis.

Three records, considerably reduced in size, obtained from one animal under the three intensities, are shown in Fig. 1. The left lateral eye in this case was functional, the others having been covered with asphaltum. In the first record it will be seen that the path bends predominantly towards the left side, there being only three places where right turns occur. Further, none of these right turns is more than 90°, while the total turning towards the left side is over 3,500°, or nearly 10 complete circles. In the second record (2,000 candle meters) the path describes over 6 complete circles to the left and less than 2 to the right, and the diameters of the circles are of larger average than those in the first record. Under the intensity of 900 candle meters the path is more irregular, describing 4.5 circles to the left and nearly 2 to the right, the diameters being much greater than in the first two records. The paths of the other 37 animals were likewise recorded and measured. A simple and exact method of expressing the amount of turning in these records is to calculate the number of degrees turned per centimeter of path, as was done by Minnich. This gives a mean figure, represented by 0° cm., which measures the angle between the circular path and a tangent to the path at any given point. Any difference in the sizes of the angles obtained from the use of the three intensities must be due to the effect of light, since all other conditions remained constant. In Table I are given some of the data showing that the degrees turned per centimeter decrease with a decrease in light intensity; *viz.*, 6.73 for 8,000 candle meters, 5.23 for 2,000, and 4.78 for 900. In other words, the diameter of the circles increases with a decrease in light intensity. Given the 0° cm. and the formula, $C = \pi D$, the diameter of the

hypothetical circle which will represent that amount of turning per centimeter may be calculated. In this way the following diameters were found: 52, 67, and 74 cm. for the intensities of 8,000, 2,000, and 900 candle meters respectively. The ratio between these diameters

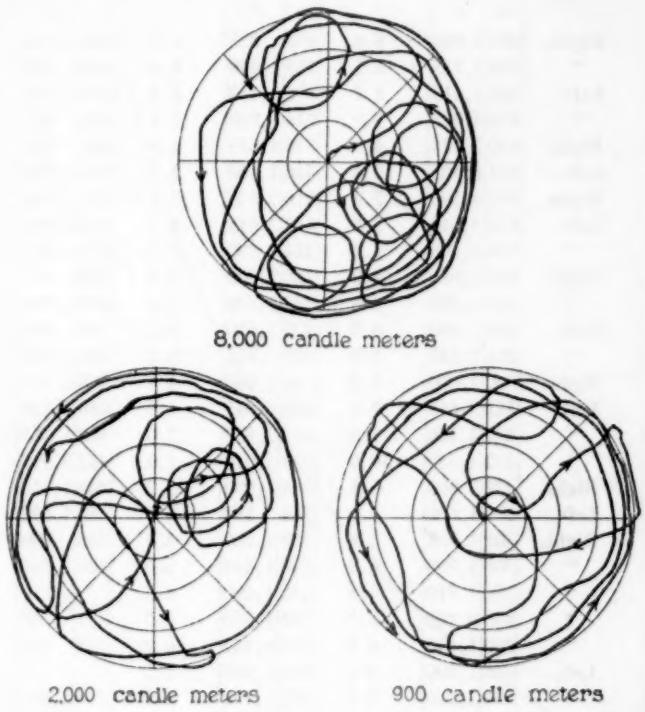


FIG. 1. Reproduced records of Animal 12, showing the pathways described under the illumination of three different intensities of light; *viz.*, 8,000 candle meters, 2,000 candle meters, and 900 candle meters. Other records from the same individual, when averaged with the ones shown here, demonstrated the number of degrees turned per centimeter to be 5.9, 4.5, and 2.4 respectively. Length of trials, 3 minutes.

is 100–128–142, which when plotted as abscissæ against candle meters as ordinates give the curve shown in Fig. 2.

If the diameters of the circles are taken to represent the effect of light upon the asymmetric animal and the ratio of these diameters is plotted against the logarithm of the photic intensity, then the result-

TABLE I.

*Length of Path, Amount of Turning, and Degrees Turned per Centimeter for Limuli under Three Intensities of Asymmetric Photic Stimulation.*

| No. | Good eye. | 8,000 candle meters. | | | 2,000 candle meters. | | | 900 candle meters. | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Length of path. | Net turn. | 0° cm. | Length of path. | Net turn. | 0° cm. | Length of path. | Net turn. | 0° cm. |
| | | *cm.* | 0° | | *cm.* | 0° | | *cm.* | 0° | |
| 9 | Right. | 664 | 3,060 | 4.6 | 476 | 825 | 1.7 | 628 | 2,690 | 4.2 |
| 10 | " | 338 | 1,735 | 5.1 | 268 | 1,440 | 5.4 | 416 | 2,565 | 6.2 |
| 11 | Left. | 404 | 2,115 | 5.2 | 372 | 1,440 | 3.9 | 260 | 1,140 | 4.4 |
| 12 | " | 476 | 2,835 | 5.9 | 424 | 1,920 | 4.5 | 408 | 981 | 2.4 |
| 13 | Right. | 460 | 2,275 | 4.9 | 346 | 1,655 | 4.8 | 208 | 810 | 3.9 |
| 14 | Left. | 584 | 6,105 | 10.4 | 536 | 4,160 | 7.7 | 596 | 1,810 | 3.0 |
| 15 | Right. | 572 | 4,190 | 7.3 | 616 | 3,135 | 5.1 | 528 | 2,380 | 4.5 |
| 16 | Left. | 612 | 3,440 | 5.6 | 664 | 3,015 | 4.5 | 496 | 1,215 | 2.4 |
| 17 | " | 528 | 3,100 | 5.9 | 424 | 1,390 | 3.3 | 476 | 1,085 | 2.3 |
| 18 | Right. | 816 | 3,980 | 4.9 | 788 | 2,390 | 3.0 | 760 | 1,530 | 2.0 |
| 19 | " | 424 | 2,500 | 5.9 | 552 | 2,550 | 4.6 | 620 | 2,560 | 4.1 |
| 20 | Left. | 380 | 2,640 | 6.9 | 428 | 2,610 | 6.2 | 240 | 1,080 | 4.5 |
| 21 | " | 264 | 2,340 | 8.9 | 308 | 1,845 | 6.0 | 386 | 1,960 | 5.1 |
| 22 | Right. | 300 | 2,090 | 7.0 | 244 | 1,080 | 4.4 | 328 | 1,170 | 3.6 |
| 23* | Left. | 512 | 3,645 | 7.1 | 400 | 1,845 | 4.6 | 456 | 2,700 | 5.9 |
| 24 | " | 420 | 3,340 | 7.9 | 412 | 2,880 | 7.0 | 300 | 1,465 | 4.9 |
| 25 | " | 432 | 4,350 | 10.0 | 528 | 3,910 | 7.4 | 432 | 3,915 | 9.0 |
| 27 | Right. | 320 | 2,090 | 6.5 | 344 | 1,800 | 5.2 | 260 | 1,350 | 5.2 |
| 28 | Left. | 300 | 1,670 | 5.6 | 264 | 180 | 0.7 | 280 | 1,080 | 3.9 |
| 29 | Right. | 308 | 1,730 | 5.6 | 288 | 1,260 | 4.4 | 208 | 990 | 4.8 |
| 30 | " | 308 | 1,930 | 6.3 | 240 | 1,440 | 6.0 | 300 | 1,540 | 5.1 |
| 31 | " | 356 | 1,710 | 4.8 | 236 | 1,080 | 4.6 | | | |
| 32 | " | 416 | 2,160 | 5.2 | 236 | 1,350 | 5.7 | 112 | 540 | 4.8 |
| 33* | " | 220 | 1,440 | 6.5 | 208 | 1,440 | 6.9 | 108 | 540 | 5.0 |
| 34 | Left. | 340 | 1,890 | 5.6 | 468 | 2,880 | 6.1 | | | |
| 35* | " | 388 | 2,260 | 5.8 | 312 | 1,170 | 3.7 | 336 | 1,800 | 5.4 |
| 36* | " | 220 | 1,460 | 6.6 | 172 | 1,080 | 6.3 | 80 | 450 | 5.6 |
| 37* | Right. | 216 | 1,260 | 5.8 | 76 | 360 | 4.7 | 128 | 720 | 5.6 |
| 38* | Left. | 212 | 2,160 | 10.0 | 220 | 1,980 | 9.0 | 276 | 2,610 | 9.5 |
| 39* | " | 204 | 1,760 | 8.6 | 180 | 1,260 | 7.0 | 144 | 540 | 3.7 |
| 40* | Right. | 356 | 2,880 | 8.1 | 256 | 1,530 | 6.0 | 384 | 2,250 | 5.8 |
| 41* | Left. | 248 | 2,700 | 10.9 | 264 | 1,980 | 7.5 | 192 | 450 | 2.3 |
| 42* | Right. | 308 | 2,660 | 8.6 | 472 | 3,060 | 6.5 | 212 | 1,890 | 8.9 |
| 43* | Left. | 84 | 720 | 8.6 | | | | | | |
| 44* | Right. | 116 | 900 | 7.8 | | | | | | |
| 45* | " | 276 | 1,800 | 6.5 | | | | | | |
| 46* | " | 376 | 2,000 | 5.3 | | | | | | |
| 47* | Left. | 464 | 3,920 | 8.5 | 312 | 2,500 | 8.0 | 300 | 1,980 | 6.6 |
| Mean | | 374 | 2,497 | 6.73±0.18 | 362 | 1,895 | 5.23±0.17 | 339 | 1,556 | 4.78±0.17 |
| Mean rate of creeping. | | 178 | | | 167 | | | 157 | | |

* Indicates trials of less than 3 minutes duration.

ing graph should be a straight line, according to the Weber-Fechner principle. In Fig. 3 this has been done, and it will be seen that the

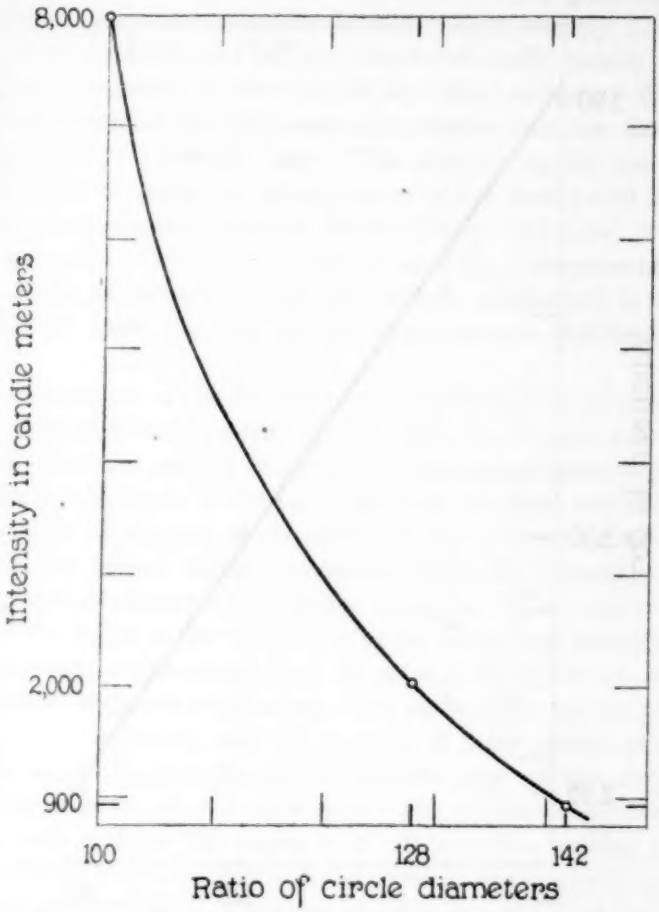


FIG. 2. Relation between the intensity of photic stimulation of asymmetric *Limuli* and the diameter of the circles turned during the circus movements. The abscissæ represent the ratios between the circle diameters, while the ordinates represent the light intensity in candle meters. Each point on the curve is the mean of 32 or more determinations.

three points lie very nearly in a straight line, and that the position of the line indicates an inverse relationship between the effect of light and its intensity, because the circle diameter is being considered, and

not the 0° cm. Because there were only three intensities used, it was not worth while to treat the graphs in Figs. 2 and 3 mathemati-

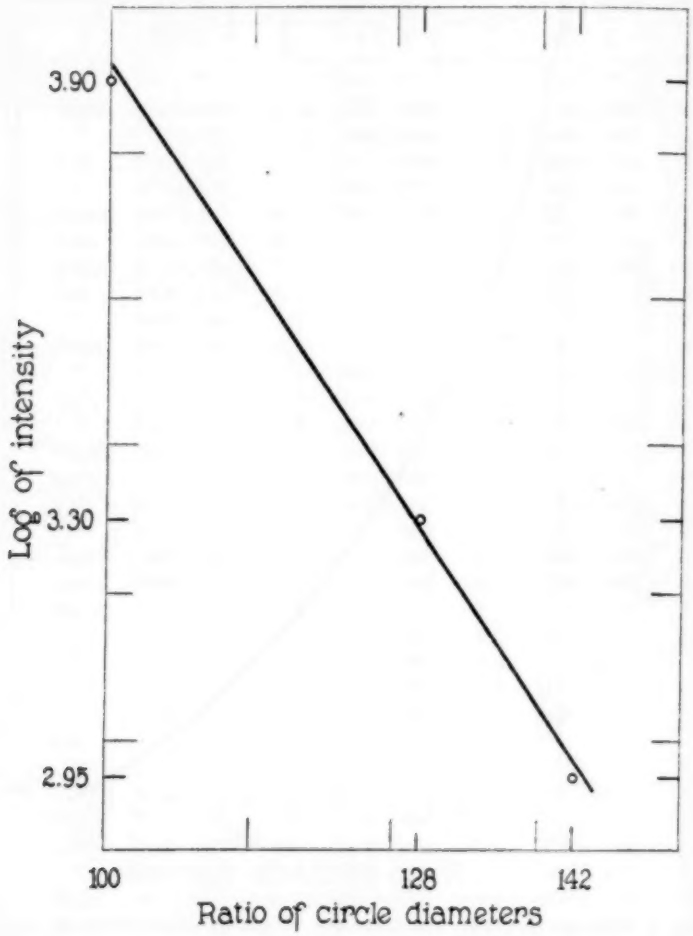


FIG. 3. Relation between the logarithm of the light intensity and the ratio of the circle diameters, showing that the circus movements of *Limulus* are performed in accordance with the Weber-Fechner law.

cally. They show, however, sufficiently clearly that the reaction of asymmetric *Limulus* is directly proportional to the intensity of the photic stimulus.

The data show another aspect of the reaction also depending upon the photic stimulus. In the last line of Table I are given the rates of creeping per minute under the three intensities; namely, 178 under 8,000,167 under 2,000, and 157 cm. under 900 candle meters. These figures are the means of the rates of individual animals. With the exceptions noted all the trials were of 3 minutes duration, the others being either 2 or 1 minute trials. This effect of varying the rate of movement by changing the photic intensity has been found in other animals, including the Japanese beetle (Moore and Cole, 1920–21) and *Drosophila* (Cole, 1922), and is another demonstration that the reactions of animals to light are entirely mechanical in nature, and depend upon the rate of the photochemical reaction in the receptor.

A consideration of the behavior of asymmetric *Limuli* under the illumination of diffuse and non-directive light leads to the conclusion, therefore, that the path of locomotion is determined immediately by the difference in tonus of the leg muscles on the right and left sides, and remotely by the rate of the photochemical reaction set up in the eye. As the animal turns a complete circle the intensity of the photic stimulus remains practically constant. The rays of light enter all the facets of the eye at any given moment of time with the same intensity, which means that all parts of the retina are equally stimulated. Impulses travel along nerve paths to the central nervous system, are coordinated, and the majority of them proceed as motor impulses to the leg muscles on the opposite side. A few may pass to the leg muscles on the same side. The difference between the number and perhaps the strength of the impulses coming to the muscles on the two sides results in the tonus of one side being greater than that of the other. Consequently the legs on the former side will move at a greater rate, and locomotion will bend towards the side on which is the functional eye. Localized retinal stimuli, as contended by Mast (1922), in such cases cannot account for the turning, since all parts of the retina are equally stimulated, even admitting that certain retinal foci are in nervous communication with definite parts of the body. It has been seen that many times this tropistic reaction is modified, so that the path does not follow a circle, or turns occasionally in the wrong direction. Such modification results from

the action of other stimuli, probably internal, acting simultaneously with the light stimulus. This has been demonstrated not only in *Limulus* but in several other animals, and there appears no reason for doubting the reports. Modifications of tropistic behavior are to be expected, and certainly do not constitute a reason for setting up a new hypothesis in place of the tropism theory.

## SUMMARY.

1. Under laboratory conditions *Limulus* from 20 to 60 mm. in diameter are positively phototropic, and execute circus movements towards the normal side, when the median and the opposite lateral eyes are removed or covered.

2. The phototropism of *Limulus* may be modified or obliterated by (*a*) fright, (*b*) hunger, (*c*) stereotropism, (*d*) photokinesis, and (*e*) unknown stimuli.

3. Quantitative measurements of the paths of animals doing circus movements demonstrate that the amount of turning varies directly with the light intensity as follows: for 8,000 candle meters the degrees turned per centimeter were 6.73; for 2,000 candle meters, 5.23; and for 900 candle meters, 4.78. In other words, the diameter of the circle varies inversely with the light intensity.

4. The rate of locomotion per minute also varies directly with the light intensity, being 178 cm. for 8,000 candle meters, 167 for 2,000, and 157 for 900.

5. These reactions are satisfactorily explained by the tropism theory.

## BIBLIOGRAPHY.

Cole, W. H., Note on the relation between the photic stimulus and the rate of locomotion in *Drosophila*, *Science*, 1922, lv, 678.

Garrey, W. E., Light and the muscle tonus of insects. The heliotropic mechanism, *J. Gen. Physiol.*, 1918–19, i, 101.

Loeb, J., Die Orientierung der Tiere gegen das Licht (tierischer Heliotropismus). Der Heliotropismus der Tiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen, Sitzungsber. phys.-med. Ges. zu Würzburg, 1888.

Mast, S. O., Photic orientation in insects, *Proc. Nat. Acad. Sc.*, 1922, viii, 240.

Minnich, D. E., The photic reactions of the honey-bee, *Apis mellifera*, L., *J. Exp. Zool.*, 1919, xxix, 343.

Moore, A. R., and Cole, W. H., The response of *Popillia japonica* to light and the Weber-Fechner law, *J. Gen. Physiol.*, 1920–21, iii, 331.

# THE BEHAVIOR OF CHLORIDES IN THE CELL SAP OF NITELLA.

By MARIAN IRWIN.

(*From the Laboratory of Plant Physiology, Harvard University, Cambridge.*)

(Received for publication, January 20, 1923.)

The writer has sought to develop direct methods for measuring penetration and exosmosis, since they are of fundamental importance in the study of permeability. In the case of *Nitella* direct determinations of chlorides can be made as follows: The end of a long cell, 4 or 5 inches in length, is cut off and the sap is gently pressed out on to a glass slide. When this is properly done a clear liquid free from chlorophyll granules is secured. This is taken up into a very fine capillary tube until it reaches a point marked on the surface. The sap is then blown out of the tube into a small white porcelain dish and titrated with a weak solution of $AgNO_3$ (0.002 to 0.0006 M) by counting the number of drops (delivered from a burette) which are required to change the color of the indicator (potassium monochromate). In a series of determinations of normal cells the probable error of the mean was less than 3 per cent of the mean.

The chloride content of the sap was ascertained by comparing it with NaCl 0.1 M which was treated in the same way as the cell sap. This was checked by collecting enough sap to fill a 1 cc. pipette and titrating this as described above. The average chloride content of the sap was 0.128 M.

Since the cells grow in water containing chlorides in very low concentration, the high concentration within the cell must be acquired during its growth. This accumulation of chloride can be followed by titration. The experiments will be reported in a later paper.

Attempts to increase the chloride content by placing cells for 2 days in balanced solutions (at pH 6.2) containing chlorides up to 0.128 M met with no success. Stronger solutions produced plasmolysis.

By placing cells in toxic solutions and titrating the cell sap at intervals the exosmosis of chlorides may be readily followed. It was found that no chloride came out unless the cells were injured, and the progress of injury could be followed by titration.

When a cell of *Nitella* (Woods Hole), 4 or 5 inches long, is placed in water with one end projecting above the surface, it is found that if this end be cut off a wave of injury progresses downward along the cell toward the uninjured end. This is accompanied by exosmosis of chlorides. In many cases if the cell is removed from the water within 1 or 2 minutes after the end is cut off, and if this portion is divided into several pieces and the sap from each is titrated separately, it is found that the lowest piece contains the most chloride and that the amount diminishes toward the cut end.

The experiments of the writer on chlorides and other inorganic substances, as well as on dyes, suggest that the entrance and accumulation of cations (except H) is favored by solutions of high pH value, and that of anions (except OH) by solutions of low pH value. The entrance of cations may involve simultaneous penetration of OH, while that of anions may be accompanied by penetration of H. It may also happen that the penetration of an ion involves exosmosis of another similarly charged ion.

This suggests that penetration and accumulation depend upon proteins, which, as shown by Loeb, react differently on the opposite sides of their isoelectric points. This assumption may also help to explain the exosmosis of substances from the cell into solutions of varying pH values.

## SUMMARY.

1. A method is given for determining the chloride content in a drop (less than 0.03 cc.) of the cell sap of *Nitella*.

2. Chlorides accumulate in the sap to the extent of 0.128 M; this accumulation can be followed during the growth of the cell. The chloride content does not increase when the cell is placed for 2 days in solutions (at pH 6.2) containing chlorides up to 0.128 M.

3. The exosmosis of chlorides from injured cells can be followed quantitatively. When one end of the cell is cut off a wave of injury progresses toward the other end; this is accompanied by a progressive exosmosis of chlorides.

# A NEW VISCOMETER.

By P. LECOMTE DU NOÜY.

(*From the Laboratories of The Rockefeller Institute for Medical Resear...*)

(Received for publication, December 11, 1922.)

The problem of measurement of viscosity has been dealt with by a number of investigators since the beginning of the 19th century, and the laws governing the flow of fluids in general have been established on a firm mathematical basis. In a recent publication, Bingham[1] reviews the actual state of the question. However, when it comes to the measurement of the viscosity of small quantities of liquids with great accuracy, as great as the control and measurement of temperature will permit, many difficulties are encountered. For this purpose, Ostwald[2] and Hess[3] have devised glass instruments based upon the use of capillary tubes. There is little doubt but that Poiseuille's[4] method of measurement of the rate of flow through a tube, although perfect and very accurate in the case of pure liquids and crystalloid solutions, may yield doubtful results when applied to colloidal solutions. Moreover, fine capillaries are very difficult to clean, and it is probable that the readings are not altogether independent of the pressure applied to the liquid. For these reasons, and because reliable measurements of viscosity of serum were sought, a new instrument was designed to overcome these deficiencies, and to give a high degree of accuracy with a quantity of liquid smaller than 1 cc.

The principle chosen was that of the coaxial cylinders. This principle has been used and studied since 1847 by de Saint-Venant,[5]

[1] Bingham, E. C., Fluidity and plasticity, New York, 1922. The term "viscometer" has been adopted, as proposed by Bingham, as this seems more logical and simple.

[2] Ostwald, W., Manuel pratique de mesures physico-chimiques, Paris, 1904, 291.

[3] Hess, W. R., *Arch. ges. Physiol.*, 1920, clxxx, 61.

[4] Poiseuille, J. L. M., *Compt. rend. Acad.*, 1842, xv, 1167.

[5] de Saint-Venant, unpublished memoirs, February 15, 1847; *Compt. rend. Acad.*, 1872, lxxiv.

Boussinesq,[6] Stokes,[7] and Margules,[8] but mainly by Couette,[9] who made a very careful investigation of the theoretical and practical side of the problem.

The main advantage of the principle of the coaxial cylinders is the direct proportionality between the torque, due to the friction exerted by the fluid on the inside cylinder (if the outside cylinder is rotating), and the rate of rotation. In other words, the readings are proportional to the rate; hence great facility for changing the sensitivity and increasing the range is obtained. This has been well established by Couette, who has shown that up to a certain maximum the ratio $\frac{P}{N}\left(\frac{\text{torque}}{\text{No. of revolutions}}\right)$ is constant. In his instrument, the radii of the cylinders of which were respectively 14.63 cm. and 14.39 cm., the maximum was 55 R. P. M., which corresponds to a linear speed of 4,840 cm. per minute. When this limit is attained, the proportionality no longer exists, owing to turbulent régime. For the viscosity of air, he shows that the same ratio (expressed this time as $\frac{T}{N}$, $T$ being the angle of torsion) is constant within 0.01 per cent up to 300 R. P. M. As in our instrument the average number of revolutions (for liquids such as water, alcohol, or sera, *i.e.* between $\eta = 0.003$ and $\eta = 0.0200$) is always less than 16 R. P. M., which means a linear speed of 50 cm. per minute, it is obvious that no error can arise from turbulent régime.

The formula used by Couette, which he obtained by integrating the equations of Navier,[10] is

$$M = \frac{4\pi\Omega R_1^2 R_0^2 h\,\epsilon}{R_1^2 - R_0^2}$$

$\Omega$ = angular speed of external moving cylinder of radius $R_1$.

$M$ = momentum, with respect to the axis, of the friction exerted by the fluid on a slice of height, $h$, in the inside cylinder of radius $R_2$.

---

[6] Boussinesq, Essai sur la théorie des eaux courantes, Paris, 1873, 62.

[7] Stokes, *Tr. Cambridge Phil. Soc.*, 1845, iii, 287.

[8] Margules, *Wien. Ber., 2te Abt.*, 1881, lxxxiii, 588.

[9] Couette, *J. chim. et physique*, series 6, 1890, xxi, 433. *J. physique*, series 2, 1890, ix, 414.

[10] Navier, *Mém. Acad. sc.*, 1823, vi, 389.

$\epsilon$ = coefficient of internal friction. (The generally accepted symbol is now $\eta$.) This formula expresses, in the case of his instrument, the value of the coefficient of viscosity, $\epsilon$ or $\eta$.

The two principal objections to this method are, first, the sliding of the liquid on the surface of the cylinders, and, second, the errors arising from the eccentricity of the two cylinders. We shall review them briefly.

*Sliding.*—This question has been discussed ever since the problem was stated, and the defenders of the opinion that the liquid slides have been unable so far to base their statements upon experimental evidence. The very careful experiments of Couette led him to conclude that there was no sliding at all, whatever the walls of the cylinders were made of. This is also the opinion of Bingham,[1] who quotes many others. The experiments of Brodmann,[11] who holds the opposite opinion, do not seem very convincing, since the variation of temperature, during one experiment, reached 0.4°C., and his apparatus was not as perfect theoretically as that of Couette.

*Eccentricity.*—Couette has made a careful study of the part played by the eccentricity and has obtained the formula

$$M = \frac{2\pi\epsilon\Omega R_0^2 R_1}{a} \int_{z_0}^{z_0+h} \frac{dz}{\sqrt{1-m^2}}$$

$a = R_1 - R_0$, mean thickness of liquid layer.
$m$ = relative eccentricity ($o > m > 1$).

This leads to the following conclusions, the first of which is of capital importance.

(*a*) The eccentricity does not affect the proportionality of the momentum, $M$, to the rate of rotation and to the coefficient of viscosity.

(*b*) The momentum, $M$, is minimum when $m = o$; that is, when the cylinders are coaxial.

(*c*) If the eccentricity is neglected, a very small relative error by excess is introduced, provided the centering has been realized approxi-

[11] Brodmann, C., *Ann. Physik. u. Chem.*, 1892, xlv, 159.

mately. If the axes are parallel and the eccentricity = $m$, the relative error in the evaluation of $e$ will be

$$\frac{m^2}{2}$$

$m$ being smaller than 1, the error is small. Moreover, it does not affect the relative value of measurements made in series, for, as stated under $a$ the readings will be comparable.

*Description of the Instrument.*

The pictures show at a glance the general disposition of the first model (Figs. 1 and 2). The rotating cup ordinarily used has an inside diameter of 10 mm. and the diameter of the plunger is 8 mm. 0.8 to 1 cc. of liquid covers the plunger entirely when immersed in the cup (Fig. 3). The phosphor-bronze suspension is a standard Leeds and Northrup galvanometer strip 0.002 inches in diameter, approximately 144 mm. long. In our first model, the plunger was removed by unscrewing it from the piece supporting the mirror. This will be improved upon, and the plunger will simply be hooked to its support. Behind the mirror is a light aluminum wire, carrying an aluminum foil vane, which acts as a damping device by moving in a semicircular bath filled with paraffin oil. This device is sufficient to insure a steady spot. By replacing the scale by a recording photographic camera, as used with the Einthoven galvanometer (cardiograph), a record in function of time or temperature may be obtained.

This first model was built with a fixed oil bath inside of which is the revolving table; the suspended system is mobile and can be raised by means of rack and pinion, in order to permit the cleaning, filling, and changing of the cup. Another improved model will be built, on the contrary, with a fixed suspended system, the rotating cup and its bath being so disposed as to slide out of the way. This will prevent the jarring of the suspension and insure a more stable zero position.

The bath is filled with oil. A resistance is immersed in it so as to raise its temperature by means of an outside rheostat when a constant increase of temperature is required. In order to keep the oil bath at a constant temperature different from that of the room, it was built

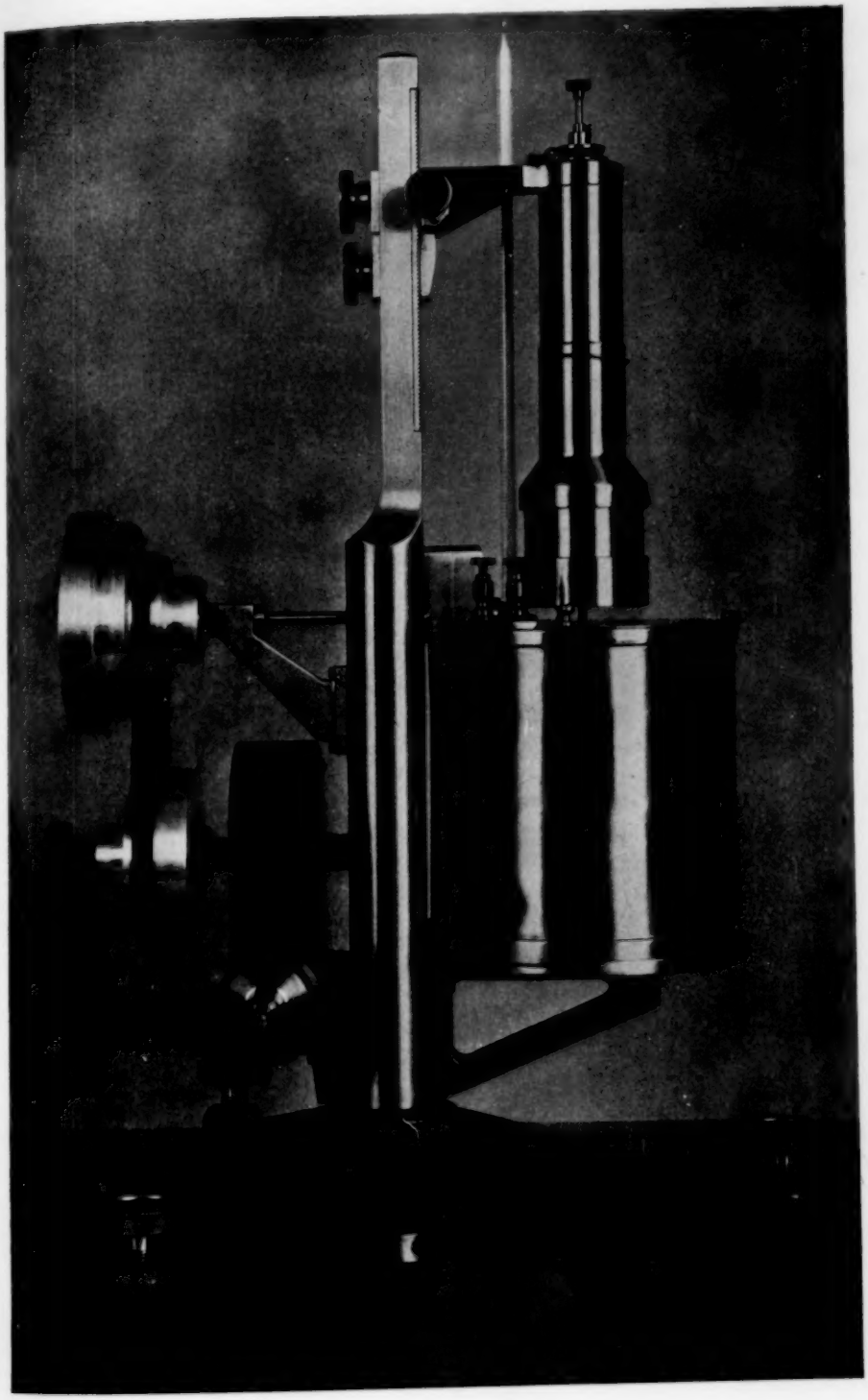

FIG. 1. View of the viscometer, mounted.

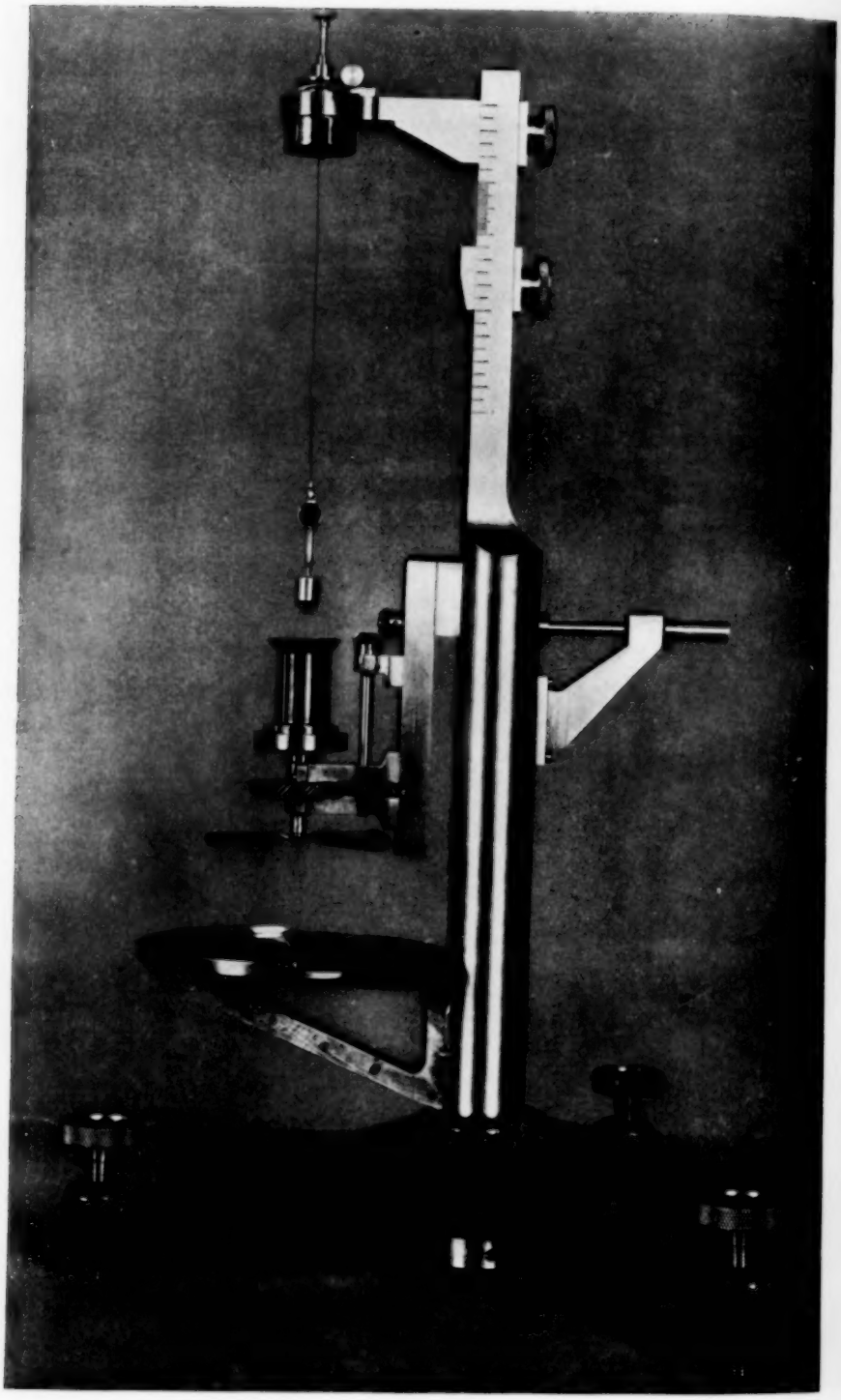

FIG. 2. Details of the suspension and rotating cup.

with double walls and a flow of water may be made to circulate between them. The temperature of the running water is regulated outside by means of any good temperature regulator, within 0.1 of a degree C. Thus, the oil bath acts as a thermic fly-wheel, and the changes of temperature in it do not amount to more than 0.01 of a degree, because the thermic inertia of the mass of oil tends to flatten the oscillations of temperature of the water, as there is no synchronism between the two. It was found convenient to heat the inflowing water by means

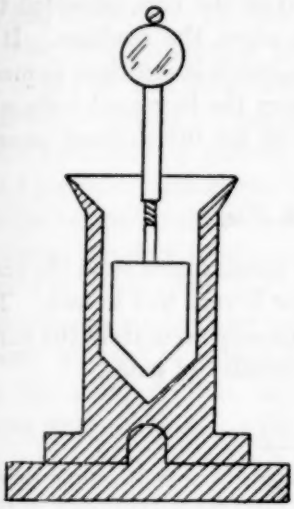

FIG. 3.

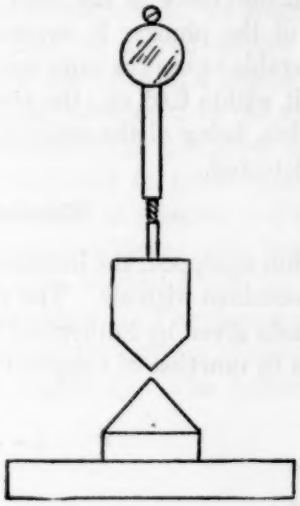

FIG. 4.

FIG. 3. Relative position of plunger and cup.

FIG. 4. In order to secure the centering of the plunger, a cone may replace the cups so that the two summits are brought into coincidence by means of the leveling screws.

of an ordinary immersion heater, and to keep the same water running through the heater, the thermoregulator, and the viscometer by means of a gear pump. The motor which keeps the cup in motion must be a constant speed motor. A constancy of 0.25 per cent is sufficient. Such motors, with speed regulators, are on the market.

In order to center the plunger and reduce the eccentricity to a minimum, a cone is placed on the revolving table instead of the cup

(Fig. 4). Then the plunger is lowered until the summits of the two cones come almost in contact. It is then very easy, by means of the leveling screws, to obtain a satisfactory coincidence of the axes. As both cones are nickel or gold plated, and highly polished, the light is reflected by them in the shape of very sharp white triangles which, when the coincidence is good, seem to be produced by two cross lines. The diameter of the threaded "tail" from which hangs the plunger is only 1 mm., which is very small with respect to the plunger, so that a slight difference in the level of the liquid in the cup, provided the top of the plunger is covered, does not affect the readings. It is preferable to use the same amount always, but it is sufficient to measure it within 0.05 cc.; the error arising from the increased surface of friction, being of the order of magnitude of the 9th decimal, cannot be detected.

*Standardization and Use.*

Thus equipped, the instrument may be standardized from the reading obtained with air. The viscosity of air is very well known. The formula given by Sutherland[12] in 1893 expresses accurately the variations in function of temperature of the viscosity of gases.

$$\eta = \eta_0 \sqrt{\frac{\Theta}{273}} \frac{1 + \frac{C}{273}}{1 + \frac{C}{\Theta}}$$

where

$\Theta$ = absolute temperature ($\theta + 273$).
$\eta_0$ = coefficient of viscosity at 0°C.
$\eta$ = coefficient of viscosity at $\theta$°C.
$C$ = Sutherland's constant (equal to 119.4 for air).

The viscosity of air at 0°C. is 0.0001733; at 24°C., it is 0.0001852; at 25°C., 0.0001858; at 26°C., 0.0001865. The temperature can be measured very accurately so that no possible error may arise from this fact. With our instruments we obtained, for a given place of the plunger in the cylinder, a deviation of 5.5 mm. on the scale at 1 m., the cup rotating at 16 R. P. M., and 109.5 mm. when the cup rotated at 320 R. P. M.; *viz.*, 20 times faster. In order to obtain closer accuracy,

[12] Sutherland, quoted by Brillouin, M., Recueil de constantes physiques, Paris, 1913, 105.

109.5 is divided by 20, thus yielding 5.48 instead of 5.5. On the scale it is impossible to make the difference between 5.5 and 5.48. Under the same conditions, water (at 16 R. P. M.) gave 264 mm. A simple ratio yields the value of $\eta$ for water at the given temperature.

$$\frac{264 \text{ (reading for water at 25°C.)}}{5.48 \text{ (reading for air at 25°C.)}} = \frac{x \text{ (viscosity of water)}}{\eta_a \text{ (viscosity of air)}} = \frac{0.008912}{0.000185}$$

0.00891 is the value obtained by the capillary tube (Poiseuille's) method as given, among others, by Thorpe and Rodger[13] in 1894. We find 0.008912. The 6th decimal may be neglected. In ordinary practise, the proportion given above may be solved readily on the slide rule and with sufficient accuracy, since 0.01 of a degree C., between 20 and 30°C., brings forth a change of 0.00002 in the value of the coefficient of viscosity (which corresponds to a relative error of 0.2 per cent); hence, the 5th decimal may only be relied upon in case the temperature can be controlled within 0.01 of a degree, and this, of course, can only be realized under special conditions. The instrument was taken apart, and when it was again ready for use, an experiment was made under very different conditions. The plunger was lowered in order to reduce the space between the bottom of the cup and the plunger, thus increasing the sensitivity. The following figures were obtained.

Temperature, 24.95°C. (oil bath).
No. of revolutions of the cup, 5 per minute.
Reading for air (on the scale), 3.9 approximately (first reading).
No. of revolutions of the cup, 30 per minute (six times faster).
Reading for air, 23 divisions ÷ 6 = 3.833 (second reading)
Coefficient of viscosity of air at 24.9 = $\eta_a$ = 0.0001857.
Reading for water (0.9 cc.), (cup rotating at 5 per minute), 182 divisions.

Hence, $\frac{182}{3.833} = \frac{\eta_w}{0.0001857}$, $\eta_w = 0.008820$.

The previous reading for water was $\eta_w = 0.008912$ at 25°C. Thus, the difference is 0.000092. The difference in temperature was 0.05°C., which should bring forth a difference of 0.0001 in the coefficient. Hence, there is a discrepancy of 0.000008 units C. G. S., which means

[13] Thorpe and Rodger, quoted by Gouré de Villemontée, G., Recueil de constantes physiques, Paris, 1913, 109.

a relative error of 0.1 per cent. As an error in the estimation of the temperature of 0.01 of a degree introduces an error of 0.2 per cent, it is obvious that this limit is an optimum which cannot be improved upon, as long as the temperature is only known within 0.01°C.

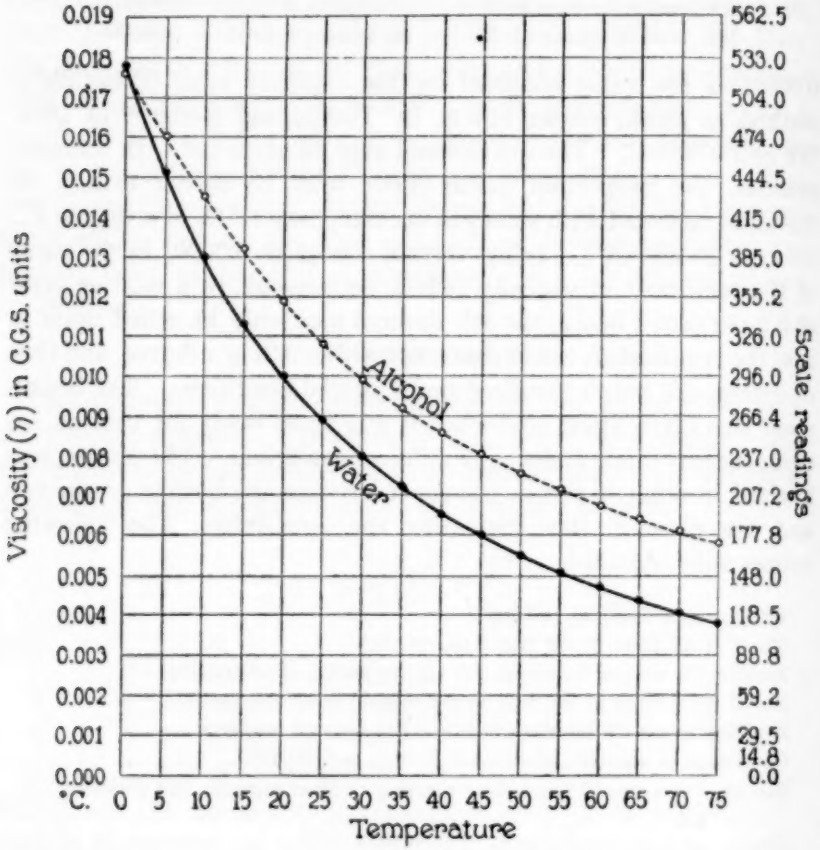


FIG. 5. Viscosity of water and alcohol in function of temperature. The values obtained with the viscometer are represented by the curve. The standard values published by Thorpe and Rodger (Poiseuille's method) are expressed by the solid black circles.

By increasing the speed of rotation and by using a larger plunger, even with the same suspension wire, a deviation of 500 divisions (full scale) may easily be obtained with air. Thus the viscosity of gases can be measured to the 7th decimal figure.

The instrument is connected with the motor by means of a set of inverted pulleys of accurately known ratio. Unless the viscosity of very viscous substances is sought, the ratios 1, 2, 6, and 12 were found to give a large range, sufficient for the average laboratory (roughly from 0.0001 to 0.1, or from 0.01 up to 10 times the viscosity of water).

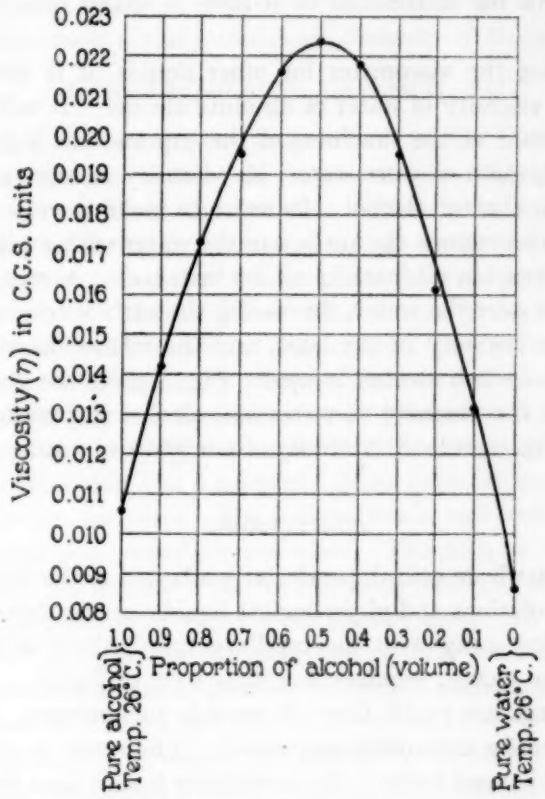


FIG. 6. Viscosity of a mixture of water and alcohol at 26°C.

To extend the range, it is only necessary to change the ratio by choosing another set of pulleys. In order to control the ratio exactly, flat pulleys are used instead of grooved ones. Gears were avoided as they are much more complicated when it comes to changing the ratio, more noisy, and do not always run as smoothly. A flat rubber belt was found to be quite satisfactory.

The coefficient of viscosity in units C. G. S. is not as frequently used in physiological laboratories as the ratio $\frac{\eta}{\eta_w}$ which may be termed specific viscosity, $\eta$ being the coefficient of viscosity of the liquid (proportional to the reading on the scale) and $\eta_w$ the coefficient of viscosity of water at the same temperature. This last figure may be measured with the instrument or it may be taken from the curve (Fig. 1).

Before using the viscometer for other liquids, it is advisable to measure the viscosity of water or absolute alcohol. It will be found that, on account of the smallness of the cup and the high value of the surface tension of pure water, its viscosity is more difficult to measure than that of alcohol. In order to make it very easy, it is only necessary to touch the surface of the water with a piece of wire previously dipped in oil (paraffin oil, for instance). A very thin layer of oil spreads over the water, decreasing the surface tension without changing the viscosity in the least, and the measurements are just as accurate as when alcohol is used. Fig. 5 gives the curve of the variations of the viscosity of water and alcohol in function of temperature. Fig. 6 gives the viscosity of a mixture of water and alcohol at 26°C.

## CONCLUSIONS.

A viscometer is described, particularly adapted to the measurement of colloidal solutions and physiological liquids (serum, blood), which, although doing away with the capillary tube (which is difficult to handle and to clean), requires less than 1 cc. of liquid.

The readings are rapid, from 30 seconds to 1 minute. The temperature is easily controlled and varied. The range is considerable and may be changed easily. The sensitivity is such that the viscosity of air can be measured to the 7th decimal, without any difficulty.

Its main advantage is that it makes possible the observation of the changes occurring in the viscosity of a liquid in function of time and temperature. It may be used as a recording viscometer by simply placing a recording photographic box before the mirror.

# THE RATE OF DECLINE OF MILK SECRETION WITH THE ADVANCE OF THE PERIOD OF LACTATION.

By SAMUEL BRODY, ARTHUR C. RAGSDALE, and CHARLES W. TURNER.

(*From the Department of Dairy Husbandry, University of Missouri, Columbia.*)

(Received for publication, December 11, 1922.)

It is well known among dairymen that lactation in the dairy cow is an orderly process with a fairly predictable average production of milk for each month of the lactation period. The milk flow rises for some time after calving, due, no doubt, to the improving condition of the animal, increased food consumption, and the perfection of the mammary gland.[1] This is followed by a steady decline until the animal goes dry. There are, of course, individual variations due to heredity and environment as regards the duration of the lactation period. The highly developed dairy cow is often milked for over a year; the beef cow goes dry as soon as the calf becomes self-supporting, which may be within 3 or 4 months. Most cows fall somewhere between these two extremes. For a given strain and breed of animal, however, there is a typical decline curve. The purpose of this article is to present the results of an investigation into the nature of this decline of milk secretion with the advance of the period of lactation. We have for this purpose compiled a very large amount of data in order to eliminate as far as possible individual variations. The data are presented in Table I and Fig. 1.

Milk secretion is, of course, a chemical or physicochemical process. Certain substances typical of blood are converted into other substances typical of milk. Like other chemical reactions the speed of milk secretion is within certain limits dependent on the supply of substances entering the reaction, for example food; on the removal of the products of the reactions, for example the removal of milk; and

[1] For other possible reasons *cf.* Osterhout, W. J. V., *Proc. Am. Phil. Soc.*, 1916, lv, 533. Osterhout, W. J. V., and Haas, A. R. C., *J. Gen. Physiol.*, 1918–19, i, 1.

probably on certain catalyzers produced by the pregnant uterus.[2] If milk secretion is a process of limited duration under a plentiful

TABLE I.

*Observed and Calculated* Values on the Decline of Milk Secretion in the Dairy Cow with the Advance of the Period of Lactation.*

| Month of lactation. | Holstein† cows (average, 95 lactation periods). Milk yield per day. | | Jersey‡ cows (average, 305 lactation periods). Milk yield per day. | | Guernsey§ cows (average, 3,215 lactation periods). Milk yield per day. | | Scrub¶ cows (average, 32 lactation periods). Milk yield per day. | |
|---|---|---|---|---|---|---|---|---|
| | Observed. | Calculated. | Observed. | Calculated. | Observed. | Calculated. | Observed. | Calculated. |
| | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* | *lbs.* |
| 1 | 39.6 | 42.9 | 30.0 | 30.8 | 33.3 | 36.1 | 20.4 | 23.3 |
| 2 | 40.3 | 40.5 | 29.4 | 29.1 | 34.3 | 33.9 | 19.0 | 19.5 |
| 3 | 38.9 | 38.4 | 27.9 | 27.4 | 32.3 | 31.9 | 16.0 | 16.4 |
| 4 | 36.5 | 36.3 | 25.9 | 25.9 | 29.9 | 30.1 | 14.0 | 13.8 |
| 5 | 33.9 | 34.3 | 24.2 | 24.5 | 28.1 | 28.3 | 12.0 | 12.8 |
| 6 | 32.5 | 32.5 | 22.7 | 23.1 | 26.4 | 26.5 | 9.7 | 9.7 |
| 7 | 30.8 | 30.7 | 21.4 | 21.8 | 24.9 | 25.1 | 8.0 | 8.1 |
| 8 | 29.3 | 29.1 | 20.6 | 20.6 | 23.7 | 23.6 | 7.0 | 6.9 |
| 9 | 27.9 | 27.5 | 19.5 | 19.4 | 22.4 | 22.2 | 5.4 | 5.7 |
| 10 | 25.4 | 26.0 | 18.6 | 18.3 | 21.0 | 20.9 | 3.7 | 4.8 |
| 11 | 23.2 | 24.6 | 17.3 | 17.3 | 19.5 | 19.7 | | |
| 12 | 20.1 | 23.3 | 17.3 | 16.3 | 17.9 | 18.5 | | |

* Values calculated from the formula $M_t = M_o e^{-kt}$, where $M_t$ = milk production during any month, $t$; $M_o$ is the theoretical value of the milk flow at the time of parturition; $e$ and $k$ have the usual meaning. The equation for the Holstein group is $M_t = 45.32e^{-0.00555t}$; for the Jersey group $M_t = 32.61e^{-0.005758t}$; for the Guernsey group $M_t = 38.3e^{-0.006051t}$; and for the Scrub group $M_t = 27.72e^{-0.01747t}$.

† Values for the Holstein cows were obtained on animals belonging to the University of Missouri herd.

‡ The data for the Jersey cows were obtained on animals belonging to dairymen in Missouri.

§ The Guernsey data were compiled from advance registry records from eastern and middle western states with a sprinkling from the Pacific Coast states.

¶ The values of the Scrub cows were interpolated from Chart 2, Bulletin 188 of the Iowa Experiment Station (1919).

[2] *Cf.* Lane-Claypon, J. E., and Starling, E. H., *Proc. Roy. Soc. London, Series B*, 1906, lxxvii, 505. O'Donoghue, C. H., *Quart. J. Micr. Sc.*, 1911–12, lvii, pt. 2, 187. Ribbert, H., *Arch. Entwcklngsmechn. Organ.*, 1898, vii, 688. Basch, K., *Deutsch. med. Woch.*, 1910, xxxvi, 987. Goltz, F., and Ewald, J. R., *Arch. ges. Physiol.*, 1896, lxiii, 362.

food supply, then there must be some factor other than food which limits the speed of the process. If the limiting reaction is chemical in nature then the decline of the speed of milk secretion should be expressed by the formula of some chemical reaction.

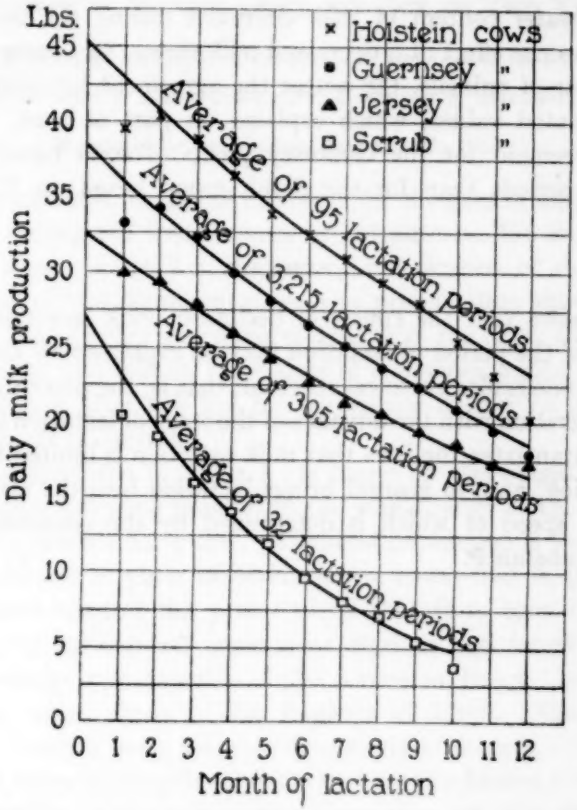


FIG. 1. The rate of decline of milk secretion with the advance of the stage of lactation. The observed values are placed along side the continuous lines of the equation $M_t = M_o e^{-kt}$.

This possibility can be tested easily by attempting to fit formulæ expressing the course of chemical reactions to the milk data. Table I and Fig. 1 show that the equation of a monomolecular chemical reaction expresses the course of decline of milk secretion with the advance of the period of lactation.

There is some deviation of the calculated from the observed values for the first month of lactation for the reasons already explained. Some deviation is also to be expected during the last month or two, due to the condition of advanced pregnancy, to the less thorough milking practiced at the end of the lactation period, and to the fact that the water content of milk decreases during the last months, which gives the effect of a decreased milk flow. Of course the larger the number of animals, the better the agreement between observed and calculated values, which explains, in part at least, the much closer agreement for the Guernsey group of cows based on 3,215 lactation periods than for the Scrub group based on 32 lactation periods.

## SUMMARY.

It is shown that the course of decline of milk secretion with the advance of the period of lactation may be expressed by the equation of a monomolecular chemical reaction; that is, the percentage decline of milk secretion with the advance of the stage of lactation is constant. This substantiates the idea that milk secretion is limited by a chemical reaction, and, in general, brings lactation into the class of processes the speed of which is determined by the concentration of a limiting substance.

# THE RATE OF GROWTH OF THE DAIRY COW.

## II. Growth in Weight after the Age of Two Years.

By SAMUEL BRODY, ARTHUR C. RAGSDALE, and CHARLES W. TURNER.

*(From the Department of Dairy Husbandry, University of Missouri, Columbia.)*

(Received for publication, January 23, 1923.)

The characteristic feature of the curve for the gain in weight of the dairy cow during the first 2 years of extrauterine life is its cyclic nature, as shown in detail in the first communication of this series.[1] The purpose of this communication is to present data showing that after the age of 2 years the course of growth of the dairy cow is non-cyclic, orderly, and follows the course of a monomolecular chemical reaction; that is, after this age the percentage decline in growth in weight with age is constant.

The data presented in Table I are based on the weights of over 14,600 animals, covering practically the whole range of the duration of life of the modern dairy cow. This cow population is very homogeneous. All are Register of Merit Jersey cows; that is, pure bred and registered cows of the Jersey breed that have been tested for production by agricultural experiment stations and found to meet certain minimum requirements of the American Jersey Cattle Club in order to admit them to the Register of Merit. The average weights of Table I were computed from these records. The data are thought to be of exceptional value because in addition to the large number of animals, the wide range of age covered, and the homogeneity of the population, the weights are not complicated by a deposition of fat, as the dairy cow shows little tendency for fattening.

The average weights for different ages shown in Table I were computed from the individual records with the aid of a correlation table (Table II). It is necessary to point out that the ages represent the time the animals started on official test, while the weights, many

[1] Brody, S., and Ragsdale, A. C., *J. Gen. Physiol.*, 1920–21, iii, 623.

TABLE I.

*Data on the Growth of the Dairy Cow (Jersey Breed).*

| Age. | No. of animals included. | Weight. | | Standard deviation. | Coefficient of variation. | Average deviation. | Deviation. |
|---|---|---|---|---|---|---|---|
| | | Computed from formula. | Mean observed weight. | | | | |
| (1) | (2) | (3) | (4) | (5) | (6) | (7) | (8) |
| *yrs.* | | *lbs.* | *lbs.* | *lbs.* | | *lbs.* | *per cent* |
| 2.25 | 3,155 | 803 | 808 ± 1.2 | 87 ± 0.74 | 10.8 ± 0.09 | 72 | 9 |
| 2.75 | 1,449 | 844 | 836 ± 1.6 | 89 ± 1.1 | 10.6 ± 0.13 | 72 | 8 |
| 3.25 | 1,523 | 875 | 872 ± 1.6 | 93 ± 1.1 | 10.7 ± 0.13 | 73 | 8 |
| 3.75 | 1,122 | 896 | 888 ± 1.9 | 92 ± 1.3 | 10.4 ± 0.15 | 72 | 8 |
| 4.25 | 1,171 | 913 | 916 ± 1.8 | 92 ± 1.3 | 10.1 ± 0.14 | 73 | 8 |
| 4.75 | 916 | 925 | 930 ± 2.2 | 100 ± 1.6 | 10.8 ± 0.17 | 76 | 8 |
| 5.5 | 1,692 | 938 | 938 ± 1.6 | 96 ± 1.1 | 10.2 ± 0.12 | 76 | 8 |
| 6.5 | 1,235 | 948 | 945 ± 1.9 | 98 ± 1.3 | 10.4 ± 0.14 | 78 | 8 |
| 7.5 | 965 | 953 | 952 ± 2.0 | 94 ± 1.4 | 9.9 ± 0.15 | 78 | 8 |
| 8.5 | 621 | 956 | 957 ± 2.6 | 97 ± 1.9 | 10.1 ± 0.19 | 79 | 8 |
| 9.5 | 364 | 958 | 962 ± 3.4 | 95 ± 2.4 | 9.9 ± 0.25 | 75 | 8 |
| 10.5 | 208 | 958.8 | 957 ± 4.4 | 94 ± 3.1 | 9.8 ± 0.32 | 76 | 8 |
| 11.5 | 108 | 959.4 | 968 ± 6.2 | 95 ± 4.4 | 9.8 ± 0.45 | 83 | 8 |
| 12.5 | 64 | 959.6 | 956 ± 6.1 | 72 ± 5.7 | 7.5 ± 0.45 | 54 | 6 |
| 13.5 | 32 | 959.8 | 961 ± 9.9 | 83 ± 7.0 | 8.6 ± 0.73 | 64 | 7 |
| 14.5 | 14 | | 1,036 ± 20.6 | 114 ± 14.5 | 11.0 ± 1.4 | 96 | 9 |
| 15.5 | 9 | | 975 ± 21.8 | 97 ± 15.4 | 10.2 ± 1.6 | 91 | 9 |
| 16.5 | 4 | | 963 ± 43.5 | 129 ± 31.0 | 13.4 ± 3.2 | 106 | 11 |

(1) The ages are mid-values of the age intervals of Table II.

(3) Computed from the formula $X = 960\ (1-e^{-0.60\,(t+\,0.77)})$, where $X$ is the weight at any birth age, $t$, in Column 1; 0.77 represents the time the fetus was carried *in utero*.

(4) Mean weight $= M = \frac{\Sigma\,(f.\,X)}{n}$. The symbols have the usual statistical meaning. Its probable error $= \pm\ 0.6745\,\frac{\sigma}{\sqrt{n}}$, where $\sigma$ is the standard deviation.

(5) Standard deviation $= \sqrt{\frac{(f.\,d^2)}{n}}$. Its probable error $= 0.6745\,\frac{\sigma}{\sqrt{2n}}$.

(6) Coefficient of variation $= C = \frac{\sigma}{M} \times 100$. Its probable error $= 0.6745\,\frac{C}{\sqrt{2n}}$.

(7) Average deviation $= A.\,D. = \frac{\Sigma\,(f.\,d)}{n}$.

(8) Percentage deviation $= \frac{A.\,D.}{M} \times 100$.

TABLE II.

*Correlation Table of Age and Weight of Jersey Cattle.*

| Weight classes. | Age classes in years. | | | | | | | | | | | | | | | | | Total. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-2.5 | 2.5-3 | 3-3.5 | 3.5-4 | 4-4.5 | 4.5-5 | 5-6 | 6-7 | 7-8 | 8-9 | 9-10 | 10-11 | 11-12 | 12-13 | 13-14 | 14-15 | 15-16 | 16-17 | |
| *lbs.* | | | | | | | | | | | | | | | | | | | |
| 400-450 | | | | | | 1 | | | | | | | | | | | | | 1 |
| 450-500 | | | | | | | | | | | | | | | | | | | |
| 500-550 | 8 | 2 | | | | | | | | | | | | | | | | | 10 |
| 550-600 | 9 | 4 | 1 | 1 | | | | | | | | | | | | | | | 15 |
| 600-650 | 124 | 23 | 14 | 4 | 4 | 1 | | 2 | 1 | | | | | | | | | | 173 |
| 650-700 | 205 | 64 | 30 | 13 | 1 | 1 | 5 | 3 | 3 | | 1 | | | | | | | | 326 |
| 700-750 | 485 | 176 | 91 | 53 | 30 | 15 | 31 | 13 | 11 | 4 | 6 | 2 | | | 1 | | | | 918 |
| 750-800 | 583 | 192 | 169 | 94 | 69 | 45 | 69 | 45 | 39 | 25 | 5 | 6 | 1 | 2 | 1 | | | | 1,345 |
| 800-850 | 837 | 377 | 345 | 224 | 204 | 142 | 199 | 146 | 102 | 64 | 31 | 22 | 13 | 2 | | 1 | 3 | 1 | 2,713 |
| 850-900 | 410 | 258 | 280 | 242 | 209 | 151 | 267 | 183 | 112 | 75 | 44 | 19 | 13 | 5 | 4 | | | | 2,272 |
| 900-950 | 333 | 222 | 328 | 244 | 272 | 212 | 422 | 274 | 217 | 143 | 78 | 58 | 29 | 22 | 7 | 3 | 1 | 2 | 2,867 |
| 950-1,000 | 89 | 72 | 123 | 117 | 166 | 128 | 246 | 201 | 169 | 88 | 72 | 29 | 12 | 18 | 8 | 2 | | | 1,540 |
| 1,000-1,050 | 56 | 48 | 99 | 91 | 141 | 136 | 269 | 231 | 185 | 142 | 76 | 41 | 22 | 12 | 9 | 1 | 4 | | 1,563 |
| 1,050-1,100 | 10 | 4 | 25 | 19 | 42 | 44 | 95 | 66 | 61 | 34 | 20 | 16 | 5 | 1 | | 3 | 1 | | 446 |
| 1,100-1,150 | 3 | 5 | 15 | 12 | 26 | 28 | 59 | 45 | 48 | 28 | 23 | 10 | 6 | | 2 | 2 | | | 312 |
| 1,150-1,200 | 2 | | 1 | 4 | 6 | 4 | 16 | 14 | 6 | 11 | 5 | 4 | 2 | 2 | | | | 1 | 78 |
| 1,200-1,250 | 1 | 1 | 2 | 3 | 1 | 7 | 9 | 7 | 9 | 5 | 2 | 1 | 5 | | | 2 | | | 55 |
| 1,250-1,300 | | | | 1 | | | 4 | 1 | | | | | | | | | | | 6 |
| 1,300-1,350 | | | | | | | 1 | 3 | 1 | 2 | | | | | | | | | 7 |
| 1,350-1,400 | | | | | | | | | | | 1 | | | | | | | | 2 |
| 1,400-1,450 | | | | | | | | | 1 | | | | | | | | | | |
| 1,450-1,500 | | 1 | | | | | | 1 | | | | | | | | | | | 2 |
| 1,500-1,550 | | | | | | 2 | | | | | | | | | | | | | 2 |
| Total..... | 3,155 | 1,449 | 1,523 | 1,122 | 1,171 | 917 | 1,692 | 1,235 | 965 | 621 | 364 | 208 | 108 | 64 | 32 | 14 | 9 | 4 | 14,653 |

of which were estimated, were sent in by the breeders at the end of the year's testing. Theoretically, therefore, a correction should be made by a whole year; practically, however, such a correction is

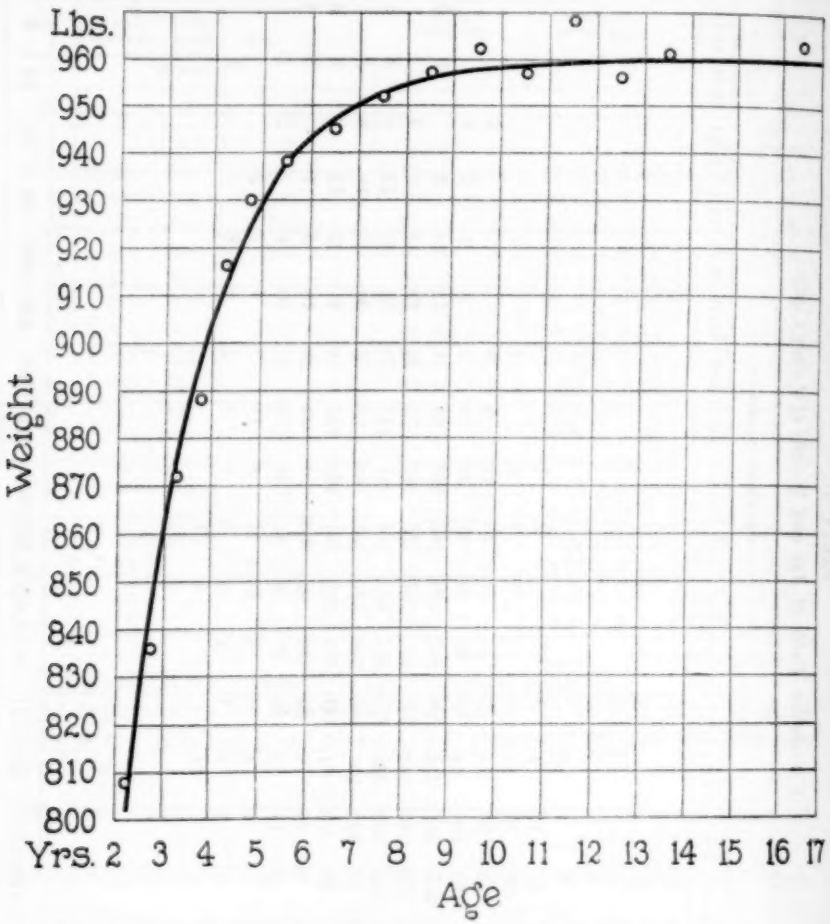


FIG. 1. Age-weight curve of the Jersey cow. The circles represent the observed values; the smooth line is the fitted curve of the equation $X = 960\ (1 - e^{-0.60(t+0.77)})$, when $X$ is the weight of the cow at any birth age, $t$.

perhaps unnecessary inasmuch as the heavy milking dairy cow seldom gains and often loses in weight during the year on official test. No correction, therefore, has been made.

The course of growth after the age of 2 years is shown in Fig. 1. In accordance with the general theory on which these studies are based, *e.g.* that growth is limited by a chemical reaction, probably of the monomolecular type, the equation of the course of a monomolecular chemical reaction was fitted to the course of growth.

The equation of a monomolecular reaction is usually written

$$X = A(1-e^{-kt})$$

In the case of growth, $A$ represents the weight of the animal at maturity, which is taken as an approximate measure of the initial concentration of the growth-limiting substance at the beginning of growth. $X$ is the weight of the animal at any time, $t$, after the beginning of growth, which is taken as an approximate measure of the amount of growth-limiting substance transformed into the product of growth at the end of time, $t$. From Fig. 1, $A$, the weight of the Jersey cow at maturity is 960 pounds. Substituting the values of $A$, $X$, and $t$, and solving for $k$, the equation becomes

$$X = 960\ (1-e^{-0.40t})$$

where $t$ is the conceptional age of the animal. In Table I weights are given in terms of birth age. For birth age $(t + 0.77)$ is written in the above equations in place of $t$, since the calf is carried *in utero* 0.77 years.

Table I and Fig. 1 show that the equation of monomolecular chemical reaction expresses very closely the course of growth in the dairy cow after the age of 2 years.

## SUMMARY.

An extensive amount of data is presented on the growth in weight of the dairy cow from 2 to 17 years of age, covering practically the entire duration of life. The data show that after the age of 2 years the rate of growth declines in a non-cyclic manner. The course of decline in growth follows the course of decline of a monomolecular chemical reaction; that is, the percentage decline in growth with age is constant.

# THE REACTION OF NEREIS VIRENS TO UNILATERAL TENSION OF ITS MUSCULATURE.

By A. R. MOORE.

(*From the Physiological Laboratory of Rutgers College, New Brunswick.*)

(Received for publication, January 23, 1923.)

In a recent paper on muscle tension and reflexes in *Lumbricus*, the writer has considered the part played by the central nervous system in reflex orientation of the head when the posterior part of the worm is passively bent. In this reaction, if the tail be bent to one side, for example to the left, the head actively turns to the right. This movement results in maintaining the line of progress parallel to the enforced orientation of the tail. For this reason the reaction has been termed the homostrophic reflex. It was found that the afferent impulses of this reflex travel through the ventral nerve cord in the forward direction only, and, further, that the efferent impulses pass to the muscles only from the anterior 15 to 20 segments, since if these segments are cut off the reflex no longer appears.[1]

It was next of interest to determine whether the homostrophic reflex occurred in the polychæte annelid, *Nereis virens*. This was accomplished best by putting the animal on a piece of filter paper wet with sea water. If, now, the tail be moved to the left, the head moves to the right, and if the tail be bent to the right, the anterior segments bend to the left. In general it can be said that passive unilateral tension in any region of the worm is followed by reflex bending of the head segments. The receptors for the reflex are therefore distributed throughout the entire length of the body.

If the ventral nerve cord is cut in the median region of the body, then impulses rising from the unilateral tension posterior to the point of section no longer pass forward to affect the orientation of the head. This proves that the ventral nerve cord is the pathway of forward conduction of the homostrophic reflex in *Nereis*. Removal of the

[1] Moore, A. R., *J. Gen. Physiol.*, 1922–23, v, 327.

head with the supraesophageal ganglion greatly weakens the reaction in its speed and definiteness, while section of the cord posterior to the subesophageal ganglion in the second or third segment abolishes any reaction to passive tension of the tail. The effector mechanism of the homostrophic reflex in *Nereis* is concerned, therefore, with a much more limited number of ganglia than in *Lumbricus*. Mediation of the reflex by the brain and the anterior two or three ventral ganglia is consistent with the high degree of cephalization in *Nereis* as compared with *Lumbricus*.

It is also important to note that in reversed locomotion in *Nereis*, as in the earthworm, an enforced asymmetrical position of the anterior segments does not affect the orientation of the tail. Indeed, it frequently happens that, in reversed locomotion, the tail will crawl across in front of the head or even over the body of the worm itself. The fact that the afferent impulses of the homostrophic reflex are propagated in one direction only, namely forward, is another proof of the existence of functional polarity in the annelid nerve cord.

It can be shown that the homostrophic reflex in *Nereis* may be weakened or even entirely masked by the interference of stereotropism. For this reason, if the tail be turned to the right and at the same time the anterior segments be gently stroked on the right side, the head may be oriented to the right, thus responding to contact and not to passive tension of the musculature of the tail, which, acting alone, would have induced orientation of the head to the left.

## SUMMARY.

1. The anterior segments of *Nereis* are oriented reflexly by passive unilateral tension of the posterior musculature.

2. The afferent impulses of the homostrophic reflex rise from any part of the worm and are conducted forward by way of the ventral nerve cord.

3. The efferent impulses flow out from the brain and anterior two or three ventral ganglia.

4. The homostrophic reflex may be partially or wholly masked by stereotropism.

# GALVANOTROPISM IN THE EARTHWORM.

By A. R. MOORE.

*(From the Physiological Laboratory of Rutgers College, New Brunswick.)*

(Received for publication, January 23, 1923.)

In a former paper Kellogg and the writer have described the effects of a galvanic current on the body positions of *Lumbricus terrestris*.[1] When the current flows transversely through such an animal or piece of one, the muscles respond by unilateral contraction on the cathodal side. The result is to bring the ends of the worm toward the cathode, thus forming a U with the median part nearest the anode.

It is not, however, necessary to have the worm submerged in the water to show galvanotropism, as the following experiment proves. A worm is put on a glass plate and the current is applied transversely through the middle of the animal by means of non-polarizable electrodes.[2] When the momentary effects of the make-shock have subsided, the head and tail actively bend toward the cathodal side although the current itself does not act directly on either end.[3] This result is shown in Fig. 1. If, now, the nerve cord is cut at a distance of a centimeter or so on either side of the locus of application of the electrodes, then the position of the part lying beyond the cut is unaffected by the action of the current. This proves that the impulses to galvanotropic bending are carried by the nerve cord and suggests that the primary action of the current is on the elements of the nerve cord.

[1] Moore, A. R., and Kellogg, F. M., *Biol. Bull.*, 1916, xxx, 131.

[2] The non-polarizable electrodes are modifications of the Schafer type. The tubes are filled with Ringer's solution and both ends are plugged with absorbent cotton. The current is obtained from a single storage cell yielding a current of 2 volts 0.1 milliampere.

[3] This experiment proves that galvanotropic orientation in the earthworm is not the result of cataphoresis of head and tail. Hence the hypothesis recently advanced by Hyman and Bellamy (Hyman, L. H., and Bellamy, A. N., *Biol. Bull.*, 1922, xliii, 313) is eliminated from consideration.

If the electrodes are placed on two opposite sides of the worm the bending of head and tail is to the cathode, as in Fig. 1; if the current is sent through the animal dorsoventrally the bending is in the dorsoventral plane, the ends of the body bending dorsally or ventrally, depending upon whether the cathode is applied respectively to the dorsal or ventral side.

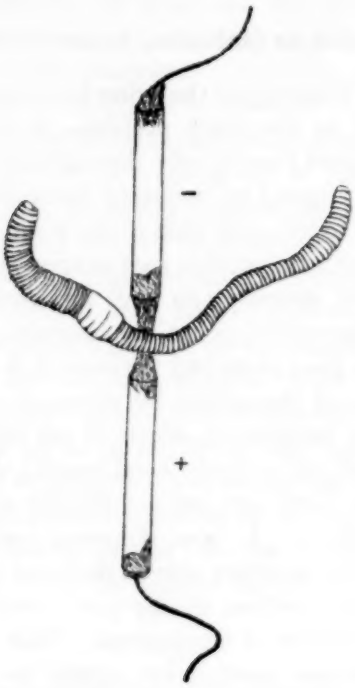


FIG. 1. A worm lying on a glass plate. The galvanic current is flowing across the animal from the ends of the modified Schafer electrodes. The sign of each electrode is indicated at the side. The worm bends both head and tail to the cathodal side.

In these experiments the current does not act directly on the ends of the animal, the parts which show the greatest displacement in orientation, but only on a few ganglia of the median segments. The differential muscle tonus, which causes bending to the cathode, is the consequence of impulses carried from the nervous elements situated in the path of the current and presumably acted upon by

it. It is to be noted that conduction of the impulses occurs equally well in the direction of either head or tail, and therefore the conducting mechanism does not exhibit polarity. This is in marked contrast to the phenomenon of forward conduction associated with the homostrophic reflex.[4]

Further evidence of the muscle tension theory of galvanotropism is furnished by experiments in which the current flows through the animal longitudinally from head to tail or the reverse. The experiments were carried out by putting the worm into a narrow trough made of paraffin. The trough was filled with tap water sufficient to cover the worm, so that the length of the column of water was 12 cm. and the cross-section was 3 sq. cm. Non-polarizable electrodes made of pads of absorbent cotton were placed at either end of the trough. The current furnished by a single storage cell was of 2 volts, 0.1 milliampere strength. In such an arrangement, if the current flows through the worm with the cathode at the head of the animal (cathodal galvanotropism), contraction of the circular musculature follows and the worm is extended to its extreme length. When the current is reversed so that the anode is at the head (anodal galvanotropism), the longitudinal muscles contract, shortening the worm during the flow of the current.

The same results are obtained if the worm is taken out of the trough and the current applied by putting the electrodes directly to the ends of the worm. Also, pieces of worm of convenient length may be used and the same results as to tonus of muscle groups obtained.

What structures are involved in these galvanotropic reactions? The influence of the cutaneous receptors can be eliminated by means of magnesium anesthesia. If this is done the reactions of the worm to the constant current are the same as in the normal worm. That the action of the current on the nerve cord alone is responsible for the galvanotropic effects described can be further demonstrated in the following way. Let a piece of worm about 2 cm. long be prepared by making first a longitudinal dorsal slit through the myodermal sheath and then removing the intestine. This leaves the muscle

[4] Moore, A. R., *J. Gen. Physiol.*, 1922–23, v, 327.

sheath and the nerve cord with connections intact. Such a preparation reacts to the current in characteristic fashion, exhibiting polarity when subjected to the longitudinal flow of the current, and unilateral muscle tone when reacting to the transverse current. If, now, the cord be removed, the tonus effects which were characteristic before have disappeared, and the same reaction is elicited whether the anode or cathode is at the anterior end of the piece. In other words, removal of the nerve cord has destroyed the galvanic polarity of the piece and left only a muscle preparation.

Since the essential action of the current is on the nerve cord, the next question is as to which elements in the cord are the seats of that action. The cord is composed of motor neurons and their fibers and dendrites. The well known fact that the axis cylinder is not stimulated by the constant current, renders it improbable that nerve fibers are the loci of action of the galvanic current. We may therefore regard the ganglion cell as the structure acted upon by the current. In fact, Loeb and Maxwell have made this assumption in their analysis of galvanotropism in *Palæmonetes*.[5]

The fact that the current causes contraction of the circular muscles only, when the cathode is at the anterior end of the animal, while with the anode at the head the longitudinal muscles alone react, must be referred to differences in the respective motor neurons relative to the direction of the galvanic current. Nernst and Barratt[6] regard the action of the electric current on nervous structures as due to the transport of ions and the subsequent blocking of them by membranes. As a result, one region of the cell has a higher concentration of a given ion than any other part of the same cell. How, then, can an electric current flowing in one direction affect one group of neurons and not a second group, while the reversed current stimulates the second group of neurons and not the first? The simplest supposition is that the ganglion cells are asymmetric with reference to the direction of the current flow and that their different orientations in space are responsible for their different reactions to the galvanic current. Such a mechanism may be visualized in the following way (Fig. 2). The usual form of the ganglion cell is approximately oval with the

[5] Loeb, J., and Maxwell, S. S., *Arch. ges. Physiol.*, 1896, lxiii, 121.
[6] Nernst, W., and Barratt, J. O. W., *Z. Electrochem.*, 1904, x, 664.

axon or fiber growing out of one end or pole. The opposite pole may or may not be provided with dendrites. Any plane cutting the cell at right angles to the long axis of the cell, *i.e.* in equatorial section, is symmetrical with respect to the axis and therefore does not offer apparent possibilities of asymmetry. But the axonal pole is different in form from its opposite pole. In other words, the cell is asymmetrical with regard to its two poles. Suppose that in order for stimulation to take place the concentration of positive ions must increase at the axonal pole of the ganglion cell but that a similar increase at the opposite pole would not lead to excitation.[7] Then if all the motor ganglion cells of the circular muscles were inclined with the axonal pole toward the anterior end of the animal, and all the ganglion cells of the longitudinal musculature were inclined with the axonal poles directed posteriorly, the necessary conditions for the galvanotropic phenomena would have been fulfilled. A diagrammatic representation of this arrangement shown in Fig. 2 makes it clear that cathodal galvanotropism would involve stimulation solely of the neurons of circular musculature, and anodal galvanotropism would involve stimulation of the neurons of longitudinal musculature alone. A transverse current ($x$ to $y$ in Fig. 2) would stimulate ganglion cells, the axonal poles of which are oriented toward the cathode. Suppose that in the diagram $x$ represents the cathode, then neurons oriented as 1 and 4 would alone respond. The result would be contraction of both circular and longitudinal muscles on the cathodal side. The foregoing hypothesis is based on the assumption that there is a direct correspondence between anatomic and dynamic polarity. As a matter of fact, there is evidence for the soundness of this view in the reactions of the ciliate protozoa to the galvanic current as, for example, in the case of paramecia.

Further evidence of the rôle of the nerve cord in galvanotropism is brought out by the following experiment. Instead of subjecting the entire worm to the action of the current in the galvanic trough, let the posterior two-thirds or three-fourths of the body be held up out of the water during the flow of the current. This can be done by

[7] It would serve our purpose just as well to suppose that stimulation is the result of an opposite set of conditions; namely, excitation results from a heaping up of positive ions at the antiaxonal pole.

holding the tail with a pair of forceps. If, now, the anterior part of the body which remains under water and is therefore subject to the action of the current, is directed head toward the cathode, extreme extension of the entire animal follows as a result of contraction of the circular muscles. This reaction in no way differs from the one shown in cathodal galvanotropism as before described. If, next, the worm is rotated so that the head is directed toward the anode, the longitudinal muscles contract and the worm shortens. This reaction is often so

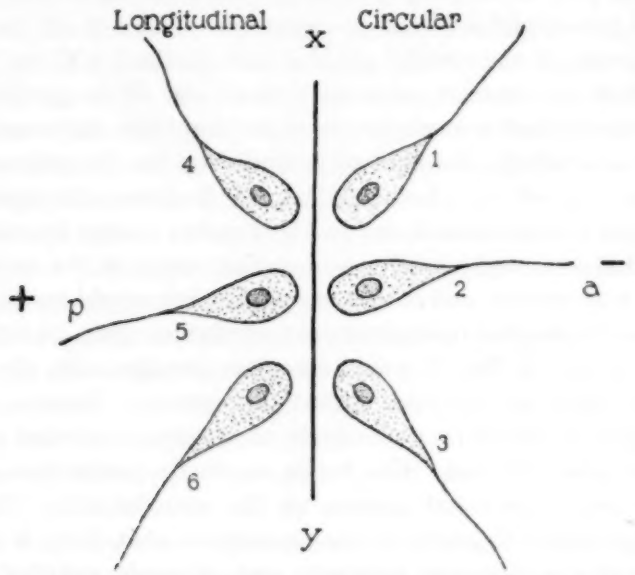


FIG. 2. Scheme showing orientation of motor ganglion cells in a ganglion. *a* denotes anterior, *p* posterior. "Longitudinal" and "circular" refer to the type of musculature innervated by the neurons figured underneath. For the sake of clearness a septum is shown drawn through the center of the ganglion, and all the ganglion cells of the circular muscles are assembled on the anterior side, while those of longitudinal musculature are put on the posterior side of the septum. In nature there is, of course, no such separation. When the current is flowing as figured, with the cathode anterior, stimulation of the neurons of circular musculature alone can take place.

strong as to cause the worm to be pulled entirely out of the trough. The result, as far as muscle tone is concerned, is identical to that observed in ordinary anodal galvanotropism.

The significant fact in these experiments is that the entire worm responds to the direction of flow of the current although only a few of the ganglia in the anterior segments are directly acted upon by the current. This proves that motor neurons of the same type of function, as for example, those of the circular muscles, are linked longitudinally by conducting tissue, as shown in the diagram of Fig. 3, so that stimulation of the ganglion cells of one type in a few anterior segments involves excitation of all the neurons of that type in the

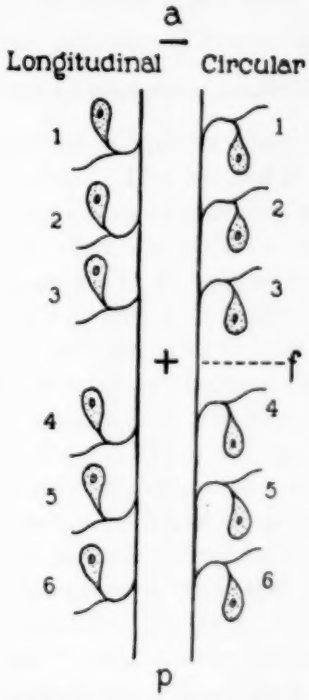


FIG. 3. Diagram showing arrangement for linear conduction of impulses between neurons having the same function. Each neuron represents a ganglion in the nerve cord. The signs are the same as in Fig. 2. With the current flowing as indicated, only ganglion cells 1, 2, and 3 of the neurons of circular musculature will be directly stimulated, but the excitation of these cells will be communicated by linear conduction to "circular" 4, 5, and 6, through the conducting fiber, *f*.

cord. The connections must, however, be strictly linear since conduction across the cord would render impossible the unilateral effects of the transverse action of the galvanic current.

# THE SELECTIVE ABSORPTION OF POTASSIUM BY ANIMAL CELLS.

## III. The Effect of Hydrogen Ion Concentration upon the Retention of Potassium.

By RALPH E. STANTON.

*(From the Biological Laboratory of Brown University, Providence.)*

(Received for publication, January 20, 1923.)

The amphoteric reaction of inactive muscle is altered during contraction to an acid reaction. Fletcher and Hopkins (1) showed that in dying muscle there is an increase in acidity up to the point of death, final loss of irritability, after which the lactic acid content remains nearly constant. Salkowski (2), as early as 1890, stated that muscle produces lactic acid only as long as it is alive.

In resting muscle the per cent of lactic acid is very small, probably close to zero (1). After a few preliminary observations, Ritchie (3) stated that resting muscle is more alkaline than pH 7.5, probably between 7.6 and 7.8. Porcelli-Titone (4), quoted by Ritchie, found a pH of 7.8 for resting skeletal muscle, and a pH of 7.2 for fatigued muscle. In 1914, Pechstein (5) found the juice of muscles stimulated to fatigue to be almost neutral, pH 6.8 to 7.0.

Marcuse (6) found an increase in lactic acid as a result of stimulating to fatigue the excised muscle of the frog. It was pointed out in the first paper of this series (7) that in such a condition of fatigue, potassium is lost in large amounts when the cells are bathed in a potassium-free Ringer solution. As much as half of the cell potassium may be lost in about 5 hours. The work by Marcuse, done in 1886, was confirmed in 1907 by Fletcher and Hopkins (1). Later work on this subject has been done recently by Ritchie, who has again shown that if muscle is stimulated to fatigue there is a slow rise in acidity.

The lactic acid produced by muscle contraction may alter the equilibrium of acids and bases within the cell. Ritchie states that the

lactic acid involved in muscle contraction is not present as such within the cell, except for a moment. It is only when an impaired condition of the muscle is brought about that an increase in acidity becomes detectable.

Gray (8) has shown that living cells, trout eggs, react to acids by taking up the hydrogen ion and releasing an equivalent amount of cation. If such cells are exposed to a KCl solution of toxic strength, a recovery may be brought about by treating the cells with an acid solution. Loeb (9) has shown that before potassium can pass through the membrane of a *Fundulus* egg, a trace of salt or acid must be present.

Inasmuch as a condition of acidity develops in a working or fatigued muscle, it was thought that hydrogen ion concentration might be the causative agent of the processes by which potassium is retained or set free in cells. The following experiments were devised to test out this possibility.

## EXPERIMENTAL.

The bull frogs used in the following experiments were caught in a swampy brook not far from the laboratory. Only healthy specimens were retained for the work. At the laboratory the frogs were well cared for, as evidenced by the fact that they remained active and in good condition. No case of red-leg developed on any of the frogs.

The frogs were caught at various times during the summer, and no frog was used that had been kept at the laboratory for over 2 or 3 months. The frogs used were practically all of the same species, *Rana catesbiana* Shaw (10). Measurements from nose to anus varied from 4 to 6 inches; from nose to toes, with legs stretched out, from 9 to 12 inches. The weight varied from about 100 to 260 gm., averaging 150 gm.

Perfusion solutions of the following composition were employed:

| | |
|---|---|
| NaCl | 6.70 gm. |
| $CaCl_2$ | 0.25 " |
| Water | to 1 liter. |

Perfusions were made through a small glass cannula in the dorsal aorta, the frogs having been previously killed by pithing.

At first some difficulty was experienced in adjusting the perfusion solutions with $NaHCO_3$ to the desired constant pH. It was found

that after short intervals, 1 hour or so, a noticeable change in the pH had taken place. For example, one solution adjusted to a pH of 7.0 changed in 1 hour to a pH of 7.6, while another changed from a pH of 7.0 to a pH of nearly 8.0 in the same time. The explanation is doubtless to be found in the fact that $CO_2$ is liberated according to the equation

$$NaHCO_3 = CO_2 + NaOH$$

The $CO_2$ escapes, leaving the solution more alkaline. In an attempt to overcome this difficulty a lot of perfusion solution was made up and adjusted to a pH of 7.0 with a 1 per cent solution of $Na_2CO_3$, in place of the customary 1 per cent $NaHCO_3$. After standing at room temperature for nearly 3 hours, the pH of the solution was still at 7.0. Consequently all perfusion solutions used in this work were adjusted by means of a 1 per cent solution of $Na_2CO_3$. After one of the 16 hour perfusions, it was found that the pH of the perfusion solution had changed only a very little, from pH 6.5 to pH 6.6.

In every case the right branch of the dorsal aorta was tied off, after the right gastrocnemius had been washed free from blood. This muscle was then removed and used as the control muscle. Perfusion was continued through the left branch of the dorsal aorta for the designated period of time. The left muscle was then removed and used as the experimental muscle. These excised muscles were dried to constant weight in ether vacuum over sulfuric acid, then digested with strong acid, and were subsequently analyzed for potassium in accord with the method of Clausen (11). All the results herein presented are based on the analyses of frog gastrocnemius muscle.

Perfusions were carried out using solutions the pH of which varied from 6.0 to 8.0. The duration of the perfusions varied from 30 minutes to 16 hours. These limits of time and of pH seem sufficient to warrant the conclusions drawn in the subsequent pages. All perfusion solutions were adjusted to the desired pH with 1 per cent solution of $Na_2CO_3$ by use of the indicators of Clark and Lubs (12). After some practice one becomes quite proficient in comparing the colors of solutions with colors shown in the chart of Clark's book.

The following six tables show, in condensed form, the results obtained in this work.

TABLE I.

*Loss of Potassium from Muscles Perfused for 30 Minutes with a Potassium-Free Ringer's Solution.*

| Sex. | pH of solution. | K in moist perfused muscle. | K in moist control muscle. | K in solids of the control muscle. | K in solids of the perfused muscle. | Apparent loss of potassium.* |
|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| F. | 6.0 | 0.291 | 0.315 | 1.685 | 1.626 | 3.50 |
| M. | 6.5 | 0.248 | 0.321 | 1.649 | 1.582 | 4.06 |
| " | 7.0 | 0.214 | 0.325 | 2.000 | 1.940 | 3.00 |
| " | 7.4 | 0.324 | 0.381 | 1.897 | 1.830 | 3.53 |
| " | 8.0 | 0.271 | 0.330 | 1.655 | 1.602 | 3.20 |

* Figures in this column are obtained by computing the difference between potassium in solids of control muscle and in those of perfused muscle as per cent of the potassium in the solids of the control muscle.

TABLE II.

*Loss of Potassium from Muscles Perfused for 1 Hour with a Potassium-Free Ringer's Solution.*

| Sex. | pH of solution. | K in moist perfused muscle. | K in moist control muscle. | K in solids of the control muscle. | K in solids of the perfused muscle. | Apparent loss of potassium. |
|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| F. | 6.0 | 0.256 | 0.313 | 1.850 | 1.737 | 6.11 |
| " | 6.5 | 0.285 | 0.378 | 1.968 | 1.858 | 5.59 |
| M. | 7.0 | 0.229 | 0.361 | 2.310 | 2.180 | 5.62 |
| " | 7.4 | 0.264 | 0.381 | 1.986 | 1.874 | 5.64 |
| F. | 8.0 | 0.279 | 0.333 | 2.025 | 1.909 | 5.72 |

TABLE III.

*Loss of Potassium from Muscles Perfused for 2 Hours with a Potassium-Free Ringer's Solution.*

| Sex. | pH of solution. | K in moist perfused muscle. | K in moist control muscle. | K in solids of the control muscle. | K in solids of the perfused muscle. | Apparent loss of potassium. |
|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| F. | 6.0 | 0.268 | 0.333 | 1.700 | 1.600 | 5.88 |
| " | 6.5 | 0.330 | 0.361 | 1.863 | 1.719 | 7.72 |
| " | 7.0 | 0.268 | 0.365 | 1.823 | 1.705 | 6.47 |
| " | 7.4 | 0.267 | 0.355 | 2.180 | 2.060 | 5.50 |
| " | 8.0 | 0.285 | 0.331 | 1.652 | 1.581 | 4.30* |
| M. | 8.0 | 0.228 | 0.336 | 1.918 | 1.813 | 5.47 |

TABLE IV.

*Loss of Potassium from Muscles Perfused for 4 Hours with a Potassium-Free Ringer's Solution.*

| Sex. | pH of solution. | K in moist perfused muscle. | K in moist control muscle. | K in solids of the control muscle. | K in solids of the perfused muscle. | Apparent loss of potassium. |
|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| F. | 6.0 | 0.285 | 0.346 | 1.784 | 1.628 | 8.74 |
| " | 6.5 | 0.272 | 0.366 | 1.899 | 1.713 | 9.79 |
| M. | 7.0 | 0.234 | 0.345 | 1.910 | 1.721 | 9.90 |
| F. | 7.4 | 0.276 | 0.365 | 1.839 | 1.703 | 7.39 |
| " | 8.0 | 0.261 | 0.348 | 1.725 | 1.572 | 8.87 |

TABLE V.

*Loss of Potassium from Muscles Perfused for 8 Hours with a Potassium-Free Ringer's Solution.*

| Sex. | pH of solution. | K in moist perfused muscle. | K in moist control muscle. | K in solids of the control muscle. | K in solids of the perfused muscle. | Apparent loss of potassium. |
|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| F. | 6.0 | 0.241 | 0.331 | 1.792 | 1.510 | 15.74 |
| " | 6.5 | 0.239 | 0.362 | 1.941 | 1.617 | 16.60 |
| " | 7.0 | 0.245 | 0.337 | 1.669 | 1.411 | 15.40 |
| " | 7.4 | 0.229 | 0.309 | 1.521 | 1.363 | 10.40* |
| " | 8.0 | 0.238 | 0.343 | 1.700 | 1.430 | 15.90 |
| M. | 7.4 | 0.235 | 0.329 | 1.660 | 1.400 | 15.66 |

TABLE VI.

*Loss of Potassium from Muscles Perfused for 16 Hours with a Potassium-Free Ringer's Solution.*

| Sex. | pH of solution. | K in moist perfused muscle. | K in moist control muscle. | K in solids of the control muscle. | K in solids of the perfused muscle. | Apparent loss of potassium. |
|---|---|---|---|---|---|---|
| | | *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| M. | 6.0 | 0.181 | 0.347 | 1.803 | 1.484 | 17.70 |
| " | 6.5 | 0.235 | 0.343 | 1.821 | 1.525 | 16.25 |
| — | 7.0 | — | — | — | — | — |
| F. | 7.4 | 0.210 | 0.317 | 1.675 | 1.387 | 17.19 |
| " | 8.0 | 0.190 | 0.327 | 1.588 | 1.333 | 16.05 |

A careful record was kept in regard to the sex of each frog in order to determine, if possible, whether or not the potassium content differed in the two sexes. The analytical results show that the amount of potassium in frog muscle apparently has no definite relation to the sex of the animal. The figures showing the per cent of potassium in the solids of fresh muscle overlap so much that no definite statement can be made. There is a tendency, however, for the figures of the male frogs to be higher than those of the female frogs.

FIG. 1. The chart is a composite of five charts (not shown here) and is presented simply to show the average loss of potassium in chart form. The separate curves, showing the loss of potassium at differing degrees of pH, are so nearly identical that this composite curve will suffice.

It will be noticed that there is a steady loss of potassium up to 8 hours, after which there is little or no loss. The remainder of the potassium is tenaciously held until the muscle becomes fatigued.

The lowest figure was found among the female frogs, 1.521 per cent; the highest among the male frogs, 2.310 per cent. The average for the females was 1.794 per cent; for the males, 1.874 per cent. According to these two figures the muscles of the male frogs are 4.26 per cent richer in potassium than those of the female frogs.

The average amount of potassium in frog muscle is 0.34 per cent, according to Fahr (13). For the species used in this work, *Rana*

*catesbiana*, the average is 0.343 per cent. This figure is based on the per cent of potassium in the fresh moist muscle, as is the figure of Fahr. The average amount of potassium in the solids of frog muscle was found to be 1.822 per cent. The minimum per cent of potassium in the fresh moist muscle was found in this work to be 0.309 per cent; the maximum was found to be 0.381 per cent. This is a difference of 18.9 per cent, and shows the necessity of expressing the per cent loss of potassium, due to perfusion, on a basis of dried solids. This is true because the per cent of water in the muscles varies even for the corresponding muscles of the same frog. As pointed out in the first paper of this series, the analytical error is small, in this work averaging 1.25 per cent.

In the two cases, starred in Tables III and V, it will be noticed that the per cent loss of potassium falls somewhat lower than the other figures in the respective columns. But it should be further noticed that the actual amount of potassium in the muscle was lower in each case than is usually found. It is also interesting to note that in each case the frog was a female. Perhaps during the production of eggs the mobile reserve of potassium in the muscle is somewhat depleted. Further work on this phase of the subject would be interesting.

At the end of each experiment the muscles showed good irritability, both to mechanical pinching of the nerves supplying the muscles and to direct and indirect electrical stimuli. After being excised the muscles were seen to quiver, thus indicating that they were far from being dead.

## SUMMARY.

By perfusing frogs for varying periods with potassium-free Ringer solutions having a pH ranging from 6.0 to 8.0, it has been determined that such solutions have little or no effect upon the retention of potassium by muscle cells.

## BIBLIOGRAPHY.

1. Fletcher, W. M., and Hopkins, F. G., *J. Physiol.*, 1906–07, xxxv, 247.
2. Salkowski, E., *Z. klin. Med.*, 1890, xvii, suppl., 97.
3. Ritchie, A. D., *J. Physiol.*, 1922, lvi, 53.
4. Porcelli-Titone, F., *Internat. Z. physik.-chem. Biol.*, 1914, i, 338.
5. Pechstein, H., *Biochem. Z.*, 1915, lxviii, 140.

6. Marcuse, W., *Arch. ges. Physiol.*, 1886, xxxix, 425.
7. Mitchell, P. H., *J. Gen. Physiol.*, 1921–22, iv, 45.
8. Gray, J., *J. Physiol.*, 1920–21, liv, 68.
9. Loeb, J., *J. Biol. Chem.*, 1916, xxvii, 339, 353, 363.
10. Dickerson, M. C., The frog book, New York, 1907, pp. 227–228, Plates lxxxvii and lxxxviii.
11. Clausen, S. W., *J. Biol. Chem.*, 1918, xxxvi, 479.
12. Clark, W. M., The determination of hydrogen ions, Baltimore, 1920.
13. Fahr, G., *Z. Biol.*, 1909, lii, 77.

# COMPARATIVE STUDIES ON RESPIRATION.

## XXIV. The Effects of Chloroform on the Respiration of Dead and of Living Tissue.

By GEORGE B. RAY.

*(From the Laboratory of Plant Physiology, Harvard University, Cambridge.)*

(Received for publication, January 22, 1923.)

One of the most interesting problems connected with the study of respiration is the action of anesthetics on the oxidations of the living cell. In order to throw some light on this the writer has made experiments on living and dead tissue.

The organism chosen was the green marine alga, *Ulva lactuca*, var. *latissima* (sea lettuce). For a number of reasons this plant is unusually desirable. It is readily obtainable at various seasons. It consists of but two layers of cells, and in consequence the gaseous exchange is rapid, which makes it favorable for experiments on respiration. There is, however, one drawback which is the result of its habitat. The plant grows in rather stagnant water and is very much contaminated with bacteria. This difficulty was in part obviated by washing the plant in running sea water for 24 hours before use. This, of course, did not remove all of the bacteria but helped to rid the plant of most of them.

Since sea water contains buffers that tend to interfere with the results of experiments by the indicator method, van't Hoff's solution was used in place of sea water.

The first experiments were on the effect of chloroform on living *Ulva*. A portion of a frond was placed in the reaction chamber of the apparatus (1), together with 100 cc. of van't Hoff's solution; a current of air free from $CO_2$ was passed through for 1 hour, and a series of readings was made to determine the normal rate. Chloroform was then added and readings were made as often as possible to determine the changes in the rate of production of $CO_2$.

The results of this experiment are shown in Fig. 1. As may be seen from the chart, 0.5 per cent chloroform (by volume) causes a continuous fall in the rate of production of $CO_2$. At the end of

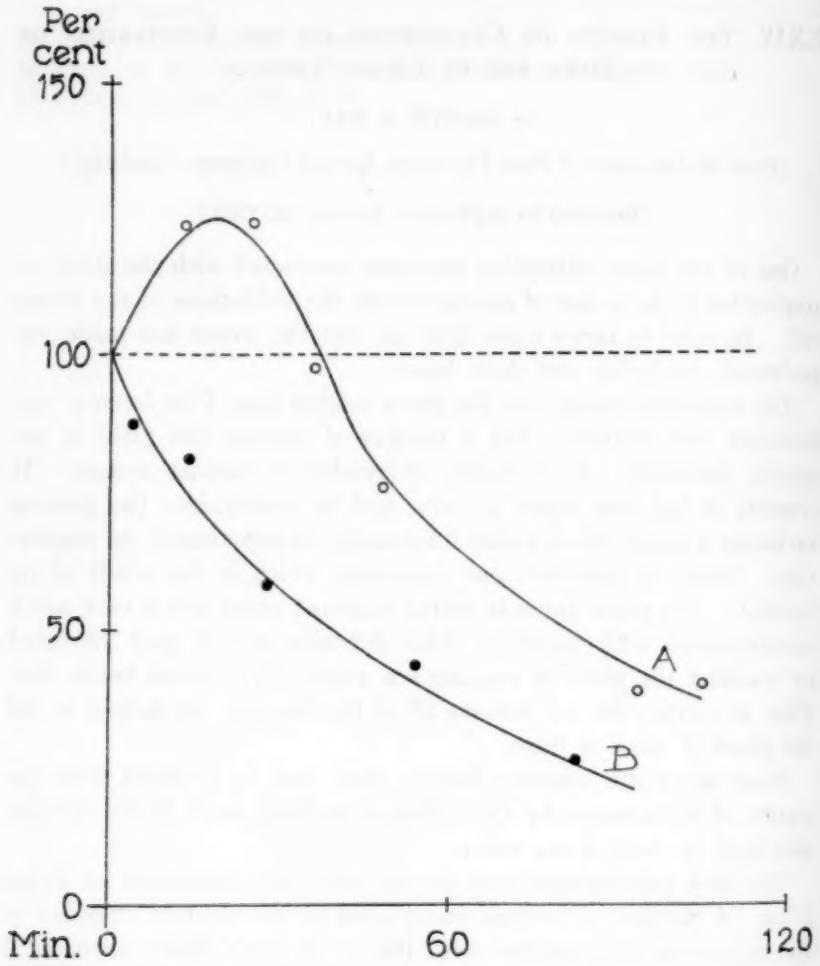


FIG. 1. Effect of chloroform 0.25 per cent (Curve A) and 0.5 per cent by volume (Curve B) on the production of $CO_2$ by *Ulva*. The normal rate is taken as 100 per cent (this is the reciprocal of the time required to make the standard change in the indicator, which varied from 2.25 to 2.75 minutes). Average of 3 experiments: probable error of the mean is less than 5 per cent of the mean.

one and one half hours the rate has dropped to 25 per cent of the normal rate. The action of 0.25 per cent is quite different. This concentration causes an increase in the rate which is followed by a decrease. These results are quite in agreement with those found by Haas (2) working with *Laminaria*.

In order to gain some insight into the mechanism of respiration, experiments were made to ascertain whether oxidation could be accelerated by the addition of substances which from a theoretical standpoint would be expected to act like the normal components of the oxidative system of the cell.

On the assumption that the first step is the formation of a peroxide in the cell, it would seem possible to influence the rate of respiration by $H_2O_2$. Accordingly, *Ulva* was placed in 100 cc. of van't Hoff's solution containing 1.0 per cent $H_2O_2$.

The result is shown in section AB of the curve given in Fig. 2. It might be expected that the $H_2O_2$ could increase the production of $CO_2$ by supplying available oxygen (3), or decrease the rate by the destruction of enzymes or other substances necessary for respiration (4). In this case the latter effect seems to predominate.

If this is the case the addition of some substance that will act like the oxidizing enzymes of the cell ought to cause *Ulva* that has been treated with $H_2O_2$ to produce $CO_2$ at an increased rate. Iron has long been known to show a remarkable resemblance to the oxidizing enzymes of the cell (5), and section BC of the curve in Fig. 2 shows that the addition of 5 cc. of 0.0005 M $Fe_2(SO_4)_3$ causes the rate to increase again.

Fig. 3 shows the effect of placing *Ulva* in van't Hoff's solution containing 5 cc. 0.0005 M $Fe_2(SO_4)_3$. There is an immediate increase in the rate of respiration, as would be expected. The subsequent fall may be due to the exhaustion of the immediately available supply of oxidizable material or to the exhaustion of the peroxide in the cell.

In order to make experiments on dead material, *Ulva* was prepared in the following manner. The material was collected, care being taken to select only the best fronds, and placed in the shade until quite dry. It was then taken into the laboratory and dried in an oven at 80°C. It was then stored in paraffined boxes until used.

Material prepared in this way was believed to be dead and when tested with gum guaiac gave no test for oxidizing enzymes, although living material gives a good test with gum guaiac.

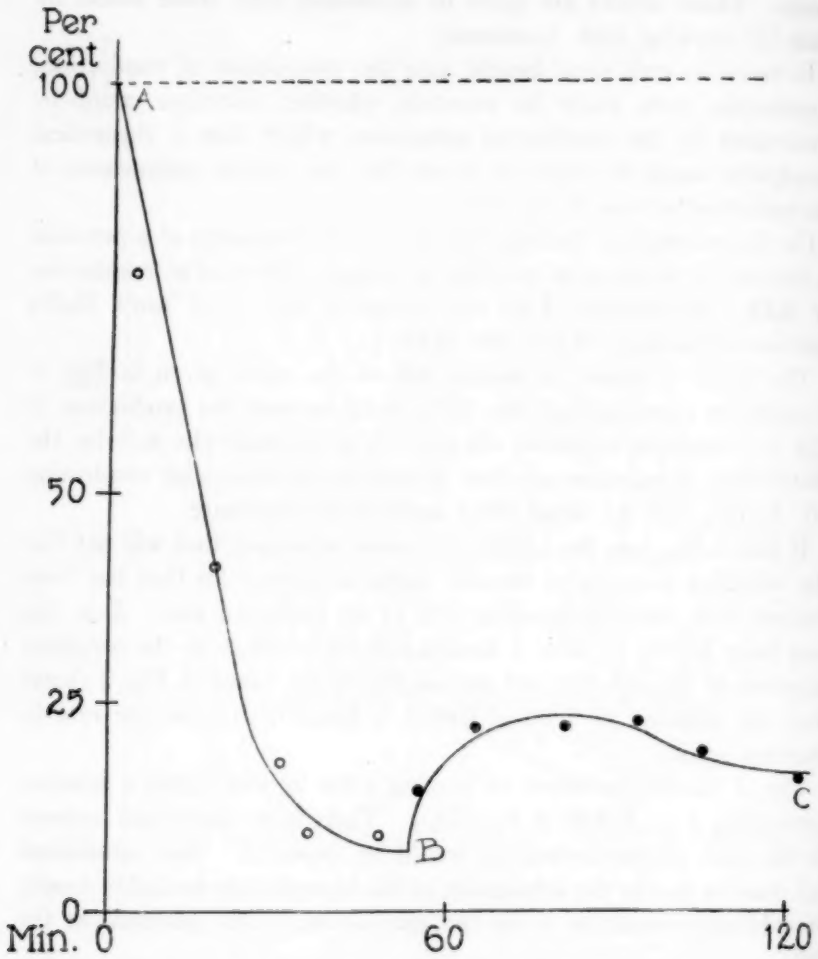


FIG. 2. The effect of 1.0 per cent $H_2O_2$ (AB) and subsequent addition of $Fe_2(SO_4)_3$ (BC). Average of 3 experiments: probable error of the mean is less than 5 per cent of the mean.

For use in the apparatus the material was dialyzed for 12 hours to remove the buffers and then placed in van't Hoff's solution. Tests showed that tissue prepared in this way did not produce $CO_2$. The

addition of $H_2O_2$ also failed to cause a production of $CO_2$. The addition of $Fe_2(SO_4)_3$, however, caused a very distinct reaction. Fig. 4

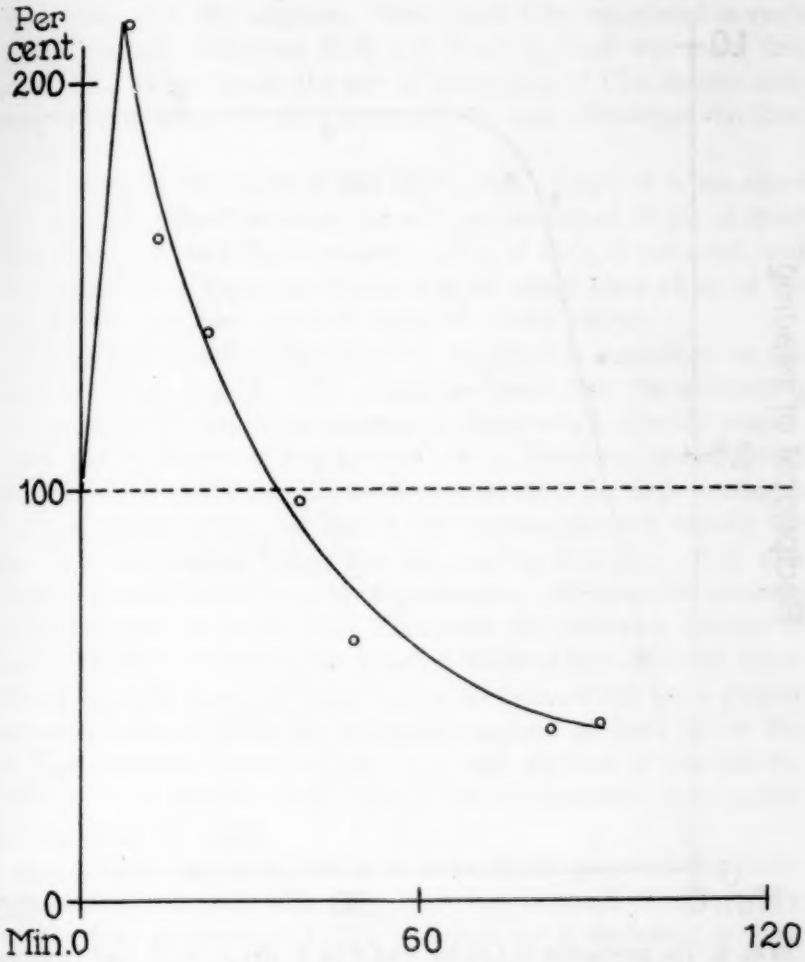


FIG. 3. The effect of the addition of $Fe_2(SO_4)_3$ (0.0005 M) to *Ulva*. Average of 3 experiments: probable error of the mean is less than 5 per cent of the mean.

shows the result of the addition of the iron solution. It may be seen that the rate of production of $CO_2$ becomes quite rapid. It was found that this rate was constant for about 6 hours and at the end of 24

hours had fallen off about 50 per cent. One might conclude from this that the peroxide and peroxidase are essential to the production of $CO_2$ by the living tissue.

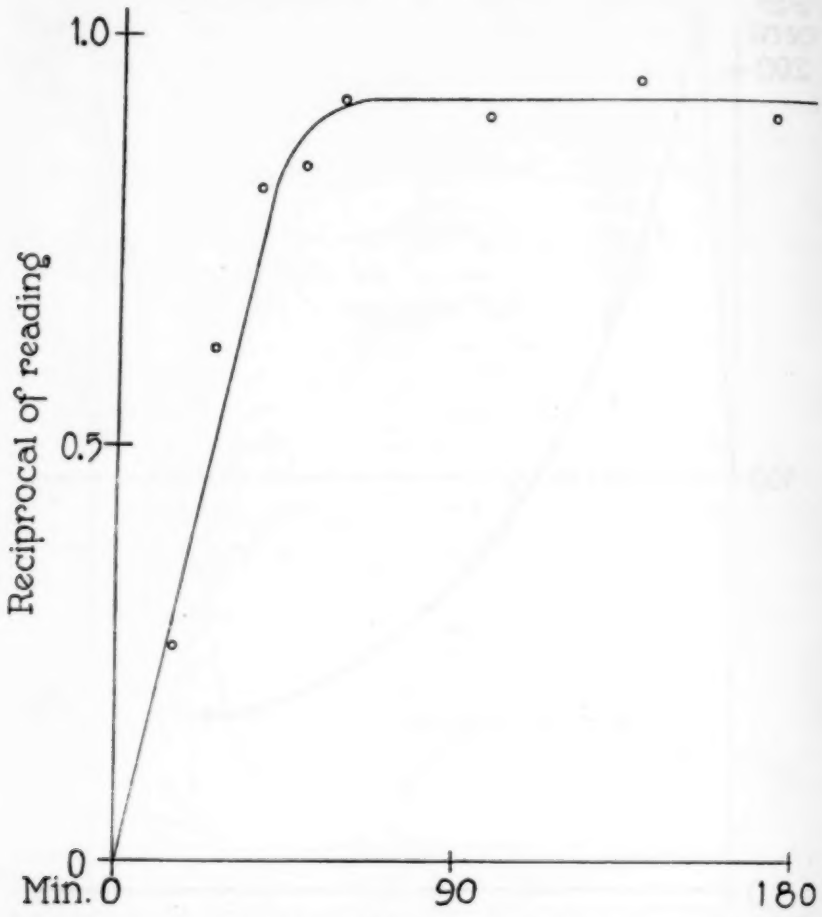


FIG. 4. The production of $CO_2$ by dead *Ulva* in 100 cc. van't Hoff's solution containing 1 per cent $H_2O_2$ when 5 cc. $Fe_2(SO_4)_3$ 0.0005 M is added. Average of 5 experiments: probable error of the mean is less than 5 per cent of the mean.

The addition of chloroform to tissue that had been treated in this manner gave results that are very interesting as compared with those of other workers who used the indicator method. It had been found

that in the case of various organisms, just as in *Ulva* (Fig. 1), certain concentrations cause a decrease in the rate, while others produce a preliminary increase. The following experiment seems to throw light upon why this happens. Some dead *Ulva* was placed in van't Hoff's solution containing $H_2O_2$ and $Fe_2(SO_4)_3$ and was made free from $CO_2$. After 1 hour the rate of production of $CO_2$ became constant (this is called 100 per cent) and 1 per cent chloroform was then added.

Fig. 5 shows the result of this experiment. Curve A is the effect of 1 per cent chloroform when the mixture consists of 20 gm. of dried *Ulva*, 75 cc. of van't Hoff's solution, 20 cc. of $H_2O_2$ (4 per cent), and 5 cc. of 0.0005 M $Fe_2(SO_4)_3$; Curve B is the effect when 10 cc. of the iron solution are used; Curve C when 20 cc. are present.

It will be noted that the shape of the curve is dependent on the amount of iron present. The writer has found that the production of $CO_2$ by dead *Ulva* in the absence of chloroform is directly proportional to the amount of iron present. It is, therefore, probable that we have to do with two consecutive processes, the first causing a rise, the second a fall, and that if the reaction proceeds rapidly the first may be finished before the first reading is taken. It is also possible that the iron inhibits the first reaction. With smaller amounts of iron the rise is observable. Thus with the minimum amount of iron (A) there is a decided rise which is followed by a decrease below 100 per cent; B shows the same sort of an increase but has a greater decrease; while C, with the maximum amount of iron (hence the greatest velocity), shows no rise upon the addition of chloroform, although it is possible that a rise would be observed if an earlier reading could be taken.

It is evident, therefore, that dead tissue in the presence of suitable reagents, when treated with chloroform, can be made to give either an increased production of $CO_2$ (followed by a decrease) or that the rate may be made to decrease from the start. Both of these effects are found in living tissue, the result depending on the concentration of the chloroform and the kind of tissue. The nature of this reaction and its chemical significance will be taken up in a later paper.

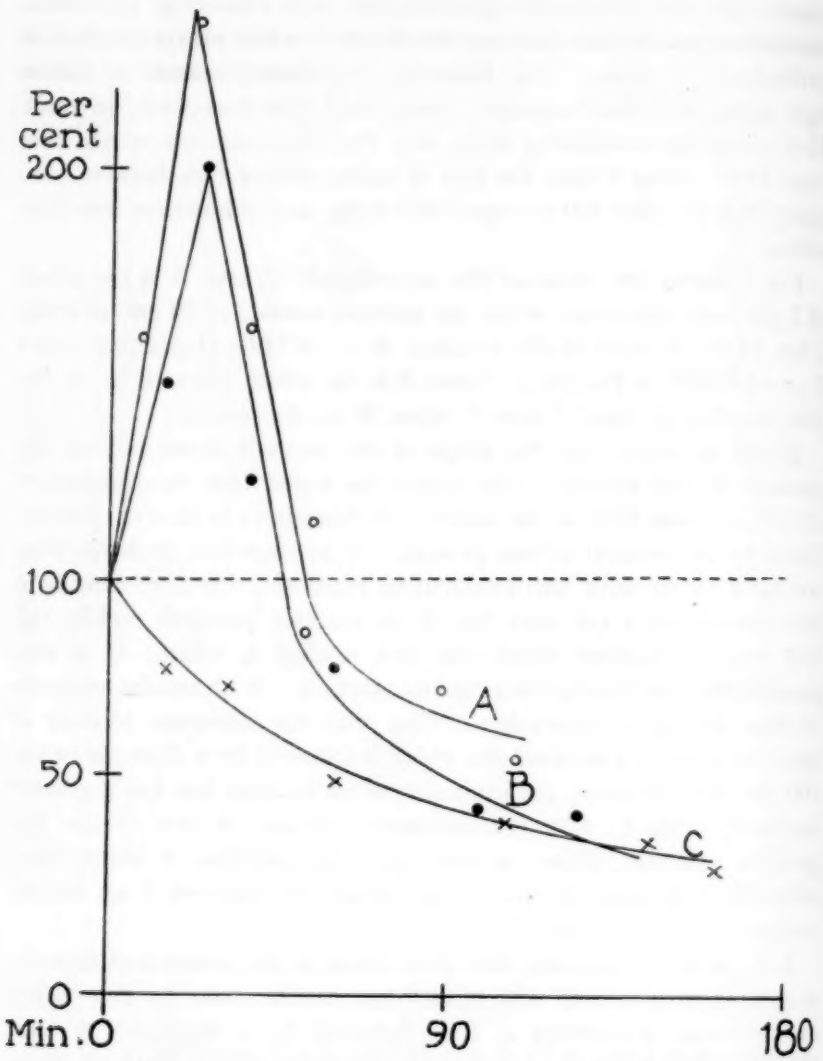


FIG. 5. The production of $CO_2$ by dead *Ulva* in 75 cc. van't Hoff's solution, 20 cc. 4 per cent $H_2O_2$, and 5 cc. (Curve A), 10 cc. (Curve B), and 20 cc. (Curve C) 0.0005 M $Fe_2(SO_4)_3$. In each case 1 per cent chloroform was added; the rate obtained in the mixture before the addition of chloroform is taken as 100 per cent. Average of 3 experiments: probable error of the mean is less than 5 per cent of the mean.

SUMMARY.

1. Chloroform in low concentration (0.25 per cent) causes an increase in the rate of production of $CO_2$ in *Ulva;* this is followed by a decrease. In higher concentration (0.5 per cent) only a decrease is observed.

2. Assuming that the normal oxidation depends on the action of peroxide and peroxidase, experiments were made by placing *Ulva* in 1.0 per cent $H_2O_2$ and in $Fe_2(SO_4)_3$ (which acts like a peroxidase). The former diminishes the rate, the latter increases and subsequently decreases it.

3. When *Ulva* is killed in such a manner as to destroy the oxidizing enzymes, no $CO_2$ is produced unless $H_2O_2$ and $Fe_2(SO_4)_3$ are present. If to this mixture chloroform is added, the effect depends on the concentration of the iron. If the concentration is low there is an increase in the production of $CO_2$ followed by a decrease. If the concentration is high the rate appears to decrease from the start.

BIBLIOGRAPHY.

1. Osterhout, W. J. V., *J. Gen. Physiol.*, 1918–19, i, 167.
2. Haas, A. R. C., *Bot. Gaz.*, 1919, lxvii, 377.
3. For references to the effect of $H_2O_2$ on oxidation in cells see Czapek, F., Biochemie der Pflanzen, Jena, 1922, iii, 753. Pfeffer, W., The physiology of plants, translated by Ewart, A. J., Oxford, 1900, i, 98, 545.
4. Bach (Bach, A., *Ber. chem. Ges.*, 1904, xxxvii, 3785) states that high concentrations of $H_2O_2$ destroy peroxidase.
5. Kastle, J. H., *Bull. Hyg. Lab., U. S. P. H.*, No. 59, 1910, 122.

# STABILITY OF SUSPENSIONS OF SOLID PARTICLES OF PROTEINS AND PROTECTIVE ACTION OF COLLOIDS.

By JACQUES LOEB.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, December 21, 1922.)

## I.

## INTRODUCTION.

While it was formerly held that the forces at the boundary between solids and liquids were purely physical, Langmuir and Harkins have come to the conclusion that these forces are primarily chemical. This viewpoint will be utilized in the problem which forms the object of this paper; namely, an investigation of the nature of the forces which keep solid particles of proteins in suspension. The large molecule of proteins does not act as a homogeneous unit, and it is necessary to discriminate for our problem between "aqueous" groups, *i.e.* groups which have a strong chemical affinity for the molecules of water (*e.g.* carboxyl, amino, or imino groups), and "oily" groups (*e.g.* hydrocarbon groups) which have a stronger affinity for each other than for water.[1] A similar view was expressed by Sheppard.[2] The molecules of gelatin in aqueous solution may adhere to each other wherever they touch each other with their "oily" groups; and if the concentration of the gelatin solution is high enough, the whole mass will set to a solid gel. In this case the affinity of the "aqueous" groups of the gelatin molecule for water need not be, and probably is not, lessened, and when the gelatin sets to a gel, the average distance between the molecules of gelatin remains the same as it was in the solution. When, however, gelatin is precipitated by a salt, the affinity of the "aqueous" groups for water is diminished and the mole-

[1] Loeb, J., Proteins and the theory of colloidal behavior, New York and London, 1922, 283.

[2] Sheppard, S. E., and Sweet, S. S., *J. Am. Chem. Soc.*, 1922, xliv, 2797.

cules attract each other over a larger area. The result is a coagulation in which the average distance between the molecules of gelatin is very much less than it was in the solution.[1] This view of the difference between gel formation and precipitation or salting out is supported by the fact observed by the writer that it requires the same high concentration of a salt to cause precipitation of a gelatin solution when it is near complete gel formation as is required when the same solution has a very low viscosity; the temperature in both cases, of course, being equal.

These facts are mentioned because they show that when a solid particle, *e.g.* collodion, is coated with a film of a protein like gelatin, it is not only *a priori* possible but, perhaps, probable that the affinity of the "aqueous" groups of the gelatin molecule for water continues to act. It is intended to show in this paper that the flocculation or precipitation of suspensions of gelatin-coated particles of collodion by salts is the same process of "salting out" by which *solutions* of gelatin in water are precipitated by salts; and it is, moreover, intended to show that the suspensions of gelatin-coated particles of collodion are as unstable at the pH of the isoelectric point of gelatin, namely 4.7, as are the solutions of gelatin, and that *suspensions* of gelatin-coated collodion particles as well as *solutions* of gelatin are stabilized at pH 4.7 by the addition of neutral salts. It follows from this that the forces which determine the stability of suspensions of gelatin-coated particles of collodion in water are the forces of chemical affinity between the "aqueous" groups of the gelatin molecule and water.

This chemical viewpoint is in strong contrast with the purely physical viewpoint, whereby suspended particles are protected against coalescence merely by their electrical double layers, which have their origin primarily in forces inherent in the water itself. On this assumption, the stability of a suspension depends only on the potential difference between the two strata of the electrical double layer surrounding the particle in water, and this P.D. is reduced to zero by comparatively low concentrations of salts, that ion of the salt being effective which has a sign of charge opposite to that of the particle. It will be shown that when collodion particles are coated with genuine crystalline egg albumin, they behave as if the film formation destroyed the affinity of the protein for water in the same way as does high

temperature ("boiling"), and in this case the chemical forces of attraction between the "aqueous" groups of albumin and water play no part in the stability of the suspensions. Such particles can be kept in suspension only by the electrical double layers surrounding the particles, as the physical theory demands.

II.

*The Nature of the Forces Which Determine the Stability of Suspensions of Gelatin-Coated Particles of Collodion.*

Suspensions of collodion particles were prepared as described in a previous publication.[3] A small quantity of such particles was put over night into 100 cc. of an aqueous solution of 0.1 per cent isoelectric gelatin at a pH of 4.7. The writer has previously shown that under such conditions a solid film of protein is formed on the surface of the collodion.[4] (It was found important not to use a higher concentration of gelatin than 0.1 per cent, since when a stronger gelatin solution is used, larger gelatin aggregates are formed to which several particles of collodion may stick. Such larger masses will settle rapidly without salt, thus rendering the test futile.) The next morning the (now gelatin-coated) collodion particles were centrifuged from the 0.1 per cent gelatin solution and then enough of the particles of a certain size were put into water of the desired pH to form a creamy suspension. 3 drops of such a stock suspension were then put at room temperature over night into test-tubes containing 10 cc. of a salt solution of a definite pH, to find out which concentration of salt was required for precipitation. The P.D. of the double electrical layer of the gelatin-coated particles is known from the cataphoretic experiments published in a preceding paper.[5]

In Table I are given the molar concentrations of different salts required to cause precipitation of the suspension of gelatin-coated collodion particles over night at room temperature.

The concentration of salts required for precipitation of the gelatin suspension bears no relation to the cataphoretic P.D., first, since the

[3] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 109.
[4] Loeb, J., *J. Gen. Physiol.*, 1919–20, ii, 577.
[5] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 395.

concentrations are far in excess of those required to depress the P.D. to any low value or even zero; and second, since there is no indication of the valency effect which is so strong in the depression of the P.D. The concentrations required to precipitate the suspension of negatively charged collodion particles *free from protein* were for NaCl, $Na_2SO_4$, $CaCl_2$, and $LaCl_3$, M/2, M/4, M/32, and M/2,048 respectively. At pH 5.8 and 11.0, the gelatin-coated particles of collodion are also negatively charged; yet the concentrations of $LaCl_3$ required for their precipitation are greater than 1 M, and for $CaCl_2$ greater than 2 M; as a matter of fact, the writer is not absolutely certain that these salts precipitate the suspension of the negatively charged gelatin-coated particles at any concentration, though this may be the case. Moreover, $Na_2SO_4$ and $Na_4Fe(CN)_6$ are more powerful precipitants for the negatively

TABLE I.

*Minimal Molar Concentrations of Salts Required to Precipitate Suspensions of Gelatin-Coated Collodion Particles Over Night at Room Temperature.*

| pH | NaCl | $CaCl_2$ | $LaCl_3$ | $Na_2SO_4$ | $Na_4Fe(CN)_6$ |
|---|---|---|---|---|---|
| | M | M | M | M | M |
| 3.0 | 2 | >2 | >1 | 1 | |
| 4.0 | >2 | >2 | >1 | 1 | 1/2 |
| 4.7 | >2 | >2 | >1 | >1/2 | 1/2 |
| 5.8 | >2 | >2 | >1 | 1 | 1/2 |
| 11.0 | >2 | 2 | | 1 | 1/2 |

charged gelatin-coated collodion particles than $LaCl_3$ or $CaCl_2$. These results admit only one explanation; namely, that the forces which determine the stability of suspensions of gelatin-coated collodion particles are not the electrical charges of the particles.

The influence of salts on the stability of suspensions of gelatin-coated particles of collodion is especially interesting when the pH of the solution is 4.7; *i.e.*, that of the isoelectric point of gelatin. The cataphoretic measurements show that at the isoelectric point, the particles are entirely uncharged. It happens that at pH 4.7 suspensions of gelatin-coated particles in water free from salt are unstable. At first sight, this would seem to suggest that electrical charges of the particles are required to make suspensions of gelatin-coated particles stable at the isoelectric point of gelatin. This assumption

is, however, refuted by the fact that comparatively low concentrations of salts, which leave the particles uncharged, make the suspensions stable. Thus M/16,000 $CaCl_2$ or M/16,000 $Na_2SO_4$ will suffice to make the suspension of gelatin-coated collodion particles stable at the isoelectric point; NaCl stabilizes the suspension in a concentration of M/512. The measurements of the cataphoretic P.D. of gelatin-coated collodion particles at the isoelectric point of gelatin show that neither NaCl nor $Na_2SO_4$ nor $CaCl_2$ causes the particles to be charged at pH 4.7, not only in concentrations as low as M/16,000 $CaCl_2$ or $Na_2SO_4$, but even in much higher concentrations.[5] Table II gives the concentrations of salts required for stabilization at the isoelectric point of gelatin.

TABLE II.

*Concentrations of Salts at Which Suspensions of Gelatin-Coated Particles of Collodion Are Stable at pH 4.7 (Isoelectric Point of Gelatin).*

| | Stable. | Precipitated. |
|---|---|---|
| NaCl | M/512 to 2 M (or higher). | 0 to M/1,024. |
| $CaCl_2$ | M/16,000 to 2 M. | 0 |
| $LaCl_3$ | M/130,000 to 1 M. | 0 |
| $Na_2SO_4$ | M/16,000 to M/2. | 0 to M/32,000 and also above M/2. |
| $Na_4Fe(CN)_6$ | M/1,000,000 to M/4. | 0 also above M/4. |

If then the forces which determine the stability of suspensions of gelatin-coated collodion particles are not the potential differences of the electrical double layer surrounding the particles, the question arises, What other forces determine this high degree of stability of these suspensions? The answer is that these forces are the same as those which keep gelatin in solution. It can be shown that the concentrations of salts required for the precipitation of suspensions of gelatin-coated particles are practically identical with those required for the salting out of gelatin from true aqueous solutions. Table III gives the minimal concentration of NaCl, $CaCl_2$, $LaCl_3$, $Na_2SO_4$, and $Na_4Fe(CN)_6$ required to "salt out" 1 per cent solutions of gelatin at pH 3.0, 4.0, 4.7 (isoelectric point), 5.8, and 11.0. The solutions were allowed to stand over night at room temperature, and were prepared in the following way.

1 gm. of originally isoelectric gelatin was dissolved in 100 cc. of water containing enough HCl or NaOH respectively to bring the solution to the desired pH and also the required concentration of salt.

The values in Table III are almost identical with the values in Table I.

It is obvious that the concentrations of salts, *e.g.* $CaCl_2$ or $LaCl_3$, required for precipitation of solutions of gelatin in water are many times greater than would be required if gelatin were in suspension; *i.e.*, if the gelatin particles were prevented from coalescing by the electrical double layers around each particle. Moreover, Tables I and III show that $Na_2SO_4$ is a better precipitant of gelatin solutions than $CaCl_2$ even if the gelatin particles are negatively charged; *i.e.*,

TABLE III.

*Molar Concentrations of Salts Required to Precipitate Gelatin from 1 Per Cent Solutions at Room Temperature.*

| pH | NaCl | $CaCl_2$ | $LaCl_3$ | $Na_2SO_4$ | $Na_4Fe(CN)_6$ |
|---|---|---|---|---|---|
| | M | M | M | M | M |
| 3.0 | 2 | >2 | >7/8 | 1/2 | |
| 4.0 | >2 | >2 | >7/8 | 1/2 or above. | 7/16 |
| 4.7 | >2 | >2 | >7/8 | 1/2 or above. | 7/16 |
| 5.8 | >2 | >2 | >7/8 | 7/8 | >7/16 |
| 11.0 | >2 | >2 | | 7/8 | 7/16 |

at pH 5.8 or at pH 11.0. If the gelatin particles were kept in solution by electrostatic charges due to electrical double layers, $CaCl_2$ should be a better precipitant than $Na_2SO_4$ when the particles of gelatin are negatively charged, which is, however, not the case. Since the concentrations of salts required for the salting out of gelatin from its aqueous solution are almost identical with the concentrations required to precipitate suspensions of gelatin-coated collodion particles, the forces determining the stability of the suspensions must be identical with those determining the stability of true solutions of gelatin, and these latter forces are forces of affinity between solute and solvent.

It can also be shown that the stabilizing effect of salts on suspensions of collodion particles coated with gelatin at the isoelectric point is due to an influence on the affinity of the protein film for water.

The writer had already shown in his book and in a preceding paper that neutral salts increase the solubility of isoelectric gelatin, and the more the higher the valency of either ion of the salt.[6] That we are dealing in this case with ordinary solubility follows from two facts; namely, first, that the time required to dissolve a given mass of solid isoelectric gelatin is diminished upon the addition of salts; and second, that the amount of isoelectric gelatin dissolved in water increases with the addition of salt. Both effects increase with the valency of one of the ions of the salt.

From the practical identity of the influence of salts on the stability of *solutions* of gelatin and of *suspensions* of gelatin-coated collodion particles, it follows that the forces which prevent the coalescence of gelatin-coated collodion particles in water are the forces determining the ordinary solubility of gelatin in water; *i.e.*, the chemical affinity between the "aqueous" groups (carboxyl, amino, or imino groups) and the molecules of water. The forces of cohesion between the "oily" groups of the gelatin molecules are no longer noticeable in the case of gelatin-coated particles of collodion.

## III.

### *Solutions of Genuine Crystalline Egg Albumin.*

Egg albumin exists in two modifications, "genuine" and "denatured" egg albumin. While genuine crystalline egg albumin is highly soluble in water, denatured egg albumin is practically insoluble. The simplest (but not the only) way to transform genuine egg albumin into its water-insoluble modification is by bringing an aqueous solution of the substance to a sufficiently high temperature. It is possible to show that the forces which keep *genuine* egg albumin in solution are those determining ordinary solubility, while the forces which keep *denatured* egg albumin in solution, or rather suspension, are the electrostatic forces of repulsion due to the electrical double layer around the particles. We will first discuss the solubility of genuine egg albumin in water.

It is understood that in all the following experiments with genuine egg albumin the temperature is ordinary room temperature not

[6] Loeb, J., *Arch. néerl. physiol. homme et animaux*, 1922, vii, 510.

exceeding 24°C., since at temperatures of 60°C. or above, crystalline egg albumin may be transformed into its insoluble modification.

Since the particles of genuine isoelectric egg albumin do not migrate at the pH of the isoelectric point, they are not kept in solution by electrical charges; moreover, the viscosity of such solutions is of so low an order of magnitude that they can contain comparatively few (if any) aggregates. The idea that crystalline egg albumin forms a true solution is also held by Sörensen[7] and is contradicted by no fact.

The concentrations of salts required to precipitate crystalline egg albumin, in the neighborhood of the isoelectric point, *i.e.* at 4.0, 4.8, and 5.8, are very high, and show no relation between the sign of

TABLE IV.

*Minimal Concentrations of Salts Required to Cause Precipitation in About 20 Hours at Room Temperature in 1 Per Cent Solutions of Originally Isoelectric Crystalline Egg Albumin at Different pH.*

| pH | NaCl | $MgCl_2$ | $CaCl_2$ | $LaCl_3$ | $Na_2SO_4$ | $Na_4Fe(CN)_6$ |
|---|---|---|---|---|---|---|
| | M | M | M | M | M | M |
| 11.0 | 1 | | 1/4 | | >3/4 | >3/8 |
| 5.8 | >2 | | >2 | 1/2 | >3/4 | >3/8 |
| 4.8 (Isoelectric point.) | >2 | | >2 | <3/4 | >3/4 | >3/4 |
| 4.0 | >2 | | 1 | 1/4 | >3/4 | >3/8 |
| 3.0 | 1 | | 1 | <1/4 | <3/4 | |
| 2.0 | 3/8 | 3/16 | | | | |

charge of the protein particle and the precipitating ion. At higher hydrogen ion concentrations, *e.g.* pH 2.0, lower concentrations of salts are sufficient for precipitation. Salts like $LaCl_3$ have a greater precipitating power for egg albumin at any pH than salts like $CaCl_2$ (Table IV).

These facts leave no doubt that the solubility of genuine crystalline egg albumin is not determined by double electrical layers surrounding the molecules or particles but is determined by the forces responsible for true crystalloidal solution, and that the precipitation of genuine egg albumin from its solution is a true "salting out" but is not caused by a diminution of the P.D. of a double layer.

[7] Sörensen, S. P. L., *Compt. rend. trav. Lab. Carlsberg*, 1915–17, xii.

IV.

## *The Stability of Suspensions of Collodion Particles Coated with Crystalline Egg Albumin.*

Collodion particles coated with genuine crystalline egg albumin do not behave like solutions of genuine egg albumin, but like suspensions of particles of denatured egg albumin; or, in other words, when genuine crystalline egg albumin forms a film on a collodion surface, it behaves as if its "aqueous" groups had ceased to react with water. Suspensions of albumin-coated collodion particles are stable only as long as the cataphoretic P.D. of the particles is above about 12 or 13 millivolts.

Collodion particles were kept over night in 1 per cent solutions of crystalline egg albumin of pH 4.8, centrifuged off from the solution, and then a milky stock suspension was prepared in water of pH 4.8. 3 drops of that suspension were added to 50 cc. of various concentrations of salt at pH 11.0, 5.8, 4.5, 4.0, and 3.0, and the velocity of migration in an electric field was measured under the microscope.

The results of these measurements are represented graphically in Figs. 1 to 5. The ordinates of the curves representing the influence of salts on the cataphoretic P.D. of the albumin-coated collodion particles are the millivolts calculated from the mobility measurements as described previously; the values are given as negative when the particle bears a negative charge. The curves are practically identical with those for the cataphoretic P.D. of particles of denatured egg albumin published in the preceding article,[5] except at pH 5.8 and 11.0, where the collodion particles coated with egg albumin behave almost like collodion particles free from albumin.

The influence of salts on the stability of suspensions of albumin-coated particles of collodion was tested in the following way. 3 drops of the stock suspension of the particles were shaken up in 10 cc. of various concentrations of salts at different pH and allowed to settle over night at room temperature. The results are given by the horizontal line "critical P.D." in Figs. 1 to 5. In all salt solutions between that line and the zero line the particles were generally precipitated over night. We shall see later that the critical P.D. for the stability of collodion particles coated with albumin is almost the same as the

critical P.D. for suspensions of particles of denatured egg albumin; namely, above 10 to 11 millivolts.

We will now go into some details. In Fig. 1 are given the cataphoretic P.D. of the albumin-coated particles of collodion at pH 4.5 at

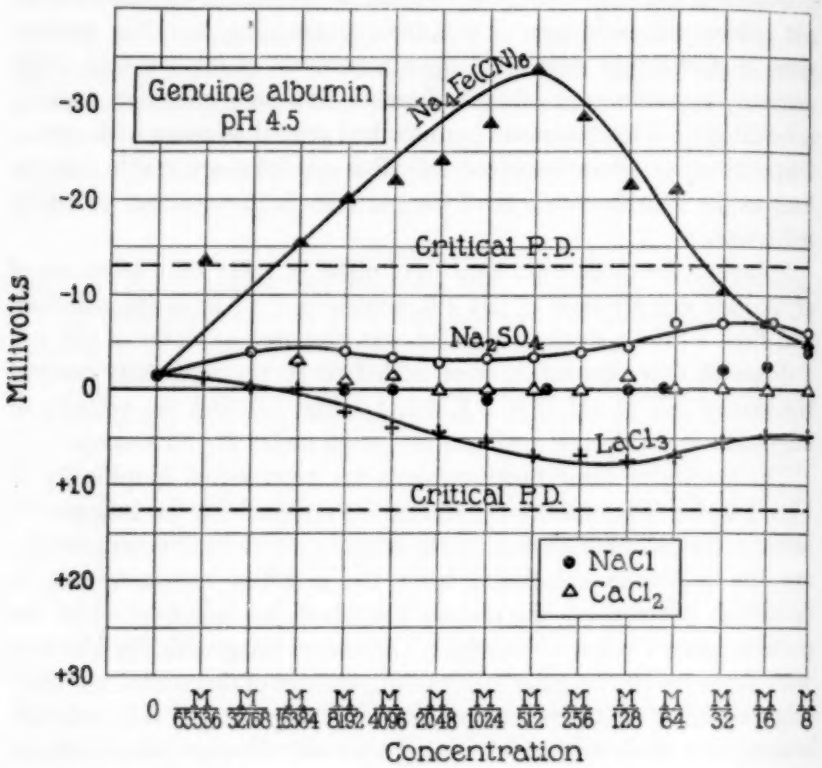


FIG. 1. Influence of salts on the cataphoretic P.D. of collodion particles coated with a film of crystalline egg albumin at pH 4.5, where the cataphoretic P.D. without salt was about zero. The two broken lines in Fig. 1 and in the following figures, with the designation "critical P.D.," give that P.D. below which the suspensions of the albumin-coated particles are no longer stable. It is obvious that only in solutions of $Na_4Fe(CN)_6$ between concentrations of M/16,000 and M/32 was the suspension stable.

which without salt the charge was zero. The isoelectric point of crystalline egg albumin is 4.8, but the cataphoretic P.D. was not zero at pH 4.8 and it was necessary to add a trace of acid to annihilate the cataphoretic migration.

The addition of NaCl, $Na_2SO_4$, $CaCl_2$, or $LaCl_3$ did not raise the cataphoretic P.D. to the critical value required for stability. $Na_4Fe(CN)_6$ in concentrations of M/16,000 or higher raised the P.D. to 13 millivolts and above, and the suspension was stable. When the

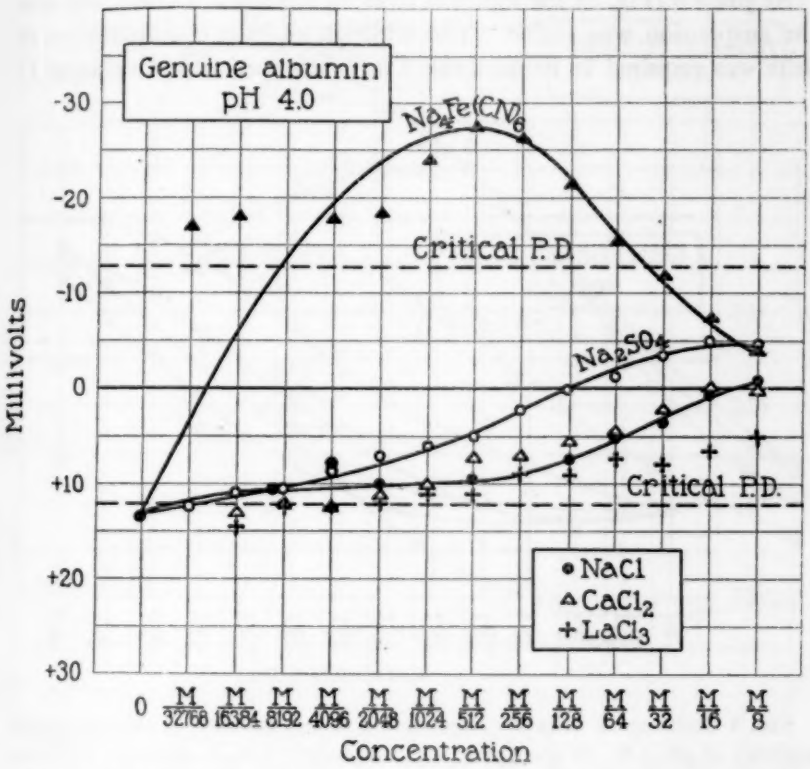


FIG. 2. Influence of salts at pH 4.0 on the cataphoretic P.D. and stability of collodion particles coated with crystalline egg albumin. Without salt the cataphoretic P.D. is +13 millivolts. $Na_4Fe(CN)_6$ brings about a reversal of the sign of charge of the particles. The suspension was stable only in solutions of $Na_4Fe(CN)_6$ between M/8,000 and M/32. In all the other cases the P.D. was below the critical value.

concentration of $Na_4Fe(CN)_6$ reached or exceeded M/32, the P.D. was depressed below the critical value and flocculation occurred.

At pH 4.0 (Fig. 2) the cataphoretic P.D. was above the critical value without salt, but the addition of $LaCl_3$, $CaCl_2$, NaCl, or $Na_2SO_4$

depressed the P.D. below that of the critical value and flocculation occurred. Stable suspensions were obtained in solutions of $Na_4Fe(CN)_6$ in concentrations between M/8,000 and M/32, because in these solutions the cataphoretic P.D. was above 13 millivolts.

At pH 3.0 (Fig. 3) the P.D. was over 30 millivolts without salt and the suspension was stable. The addition of high concentrations of salts was required to depress the P.D. below the critical value of 13

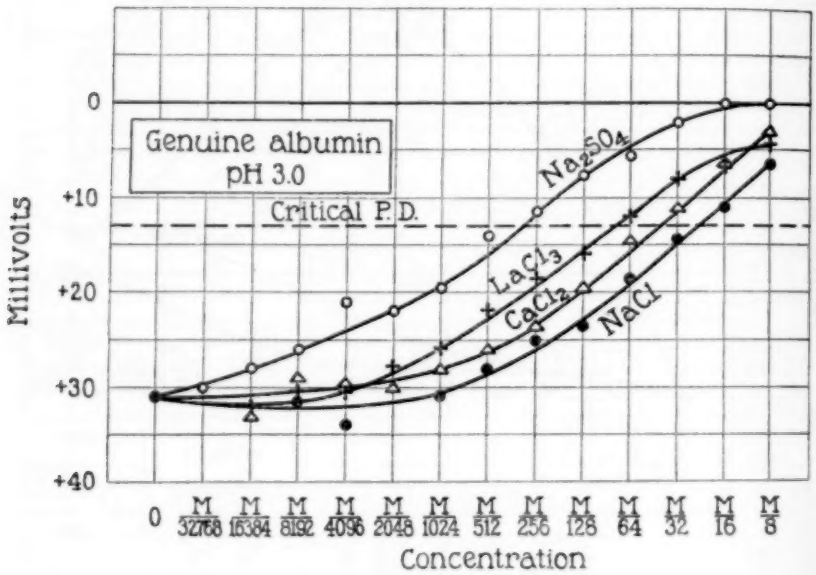


FIG. 3. Influence of salts on cataphoretic P.D. and stability of albumin-coated particles of pH 3.0. Without salt the cataphoretic P.D. is about 31 millivolts and the particles are positively charged. The suspension is stable as long as the concentration of the salt is not too high. Flocculation occurs when the values for the P.D. fall between the line for critical P.D. and the zero line.

millivolts. M/16 NaCl, M/32 $CaCl_2$, about M/64 $LaCl_3$, and M/256 $Na_2SO_4$ were required to cause flocculation.

Figs. 4 and 5 give the result of experiments at pH 5.8 and 11.0. The curves are so much like those for the P.D. of collodion particles free from protein that the suspicion exists that the albumin had been dissolved by the alkali.

Hence, collodion particles coated with genuine egg albumin form stable suspensions only by virtue of their electrical double layers; as soon as the P.D. of the double layer falls below a critical value, the suspension is no longer stable. The particles attract each other in

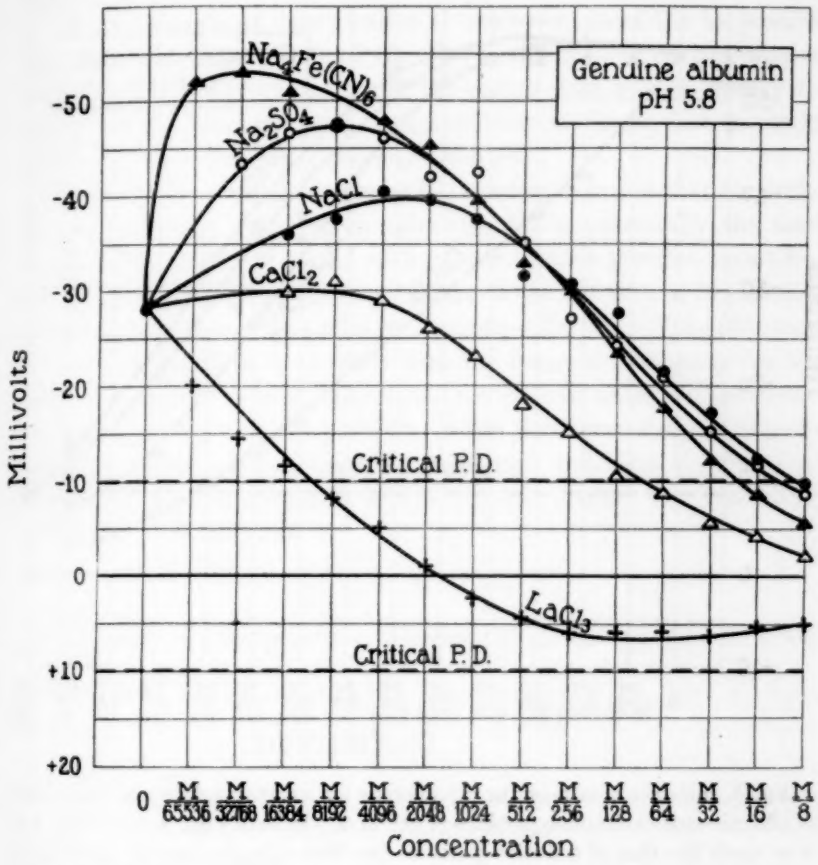


FIG. 4. Influence of salts on the cataphoretic P.D. and the stability of suspensions of albumin-coated collodion particles at pH 5.8.

the same way as do the particles of denatured egg albumin when the P.D. falls below the critical value of about 12 millivolts.

It is difficult to understand why genuine egg albumin when it forms a solid film on collodion particles should lose its solubility in water

and behave in that respect like boiled egg albumin, yet the fact that albumin is denatured when forming a film is supported by the observations of Ramsden.[8] This author found that proteins have a tendency to form a solid film at the surface of liquids on account of their

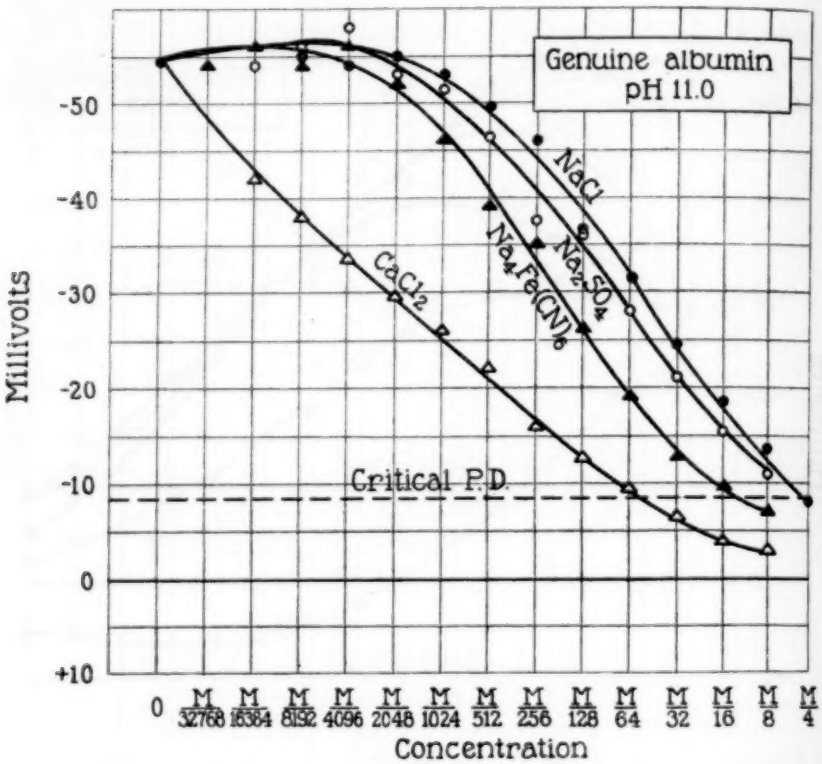


FIG. 5. Influence of salts on the cataphoretic P.D. and the stability of suspensions of albumin-coated collodion particles at pH 11.0. In both Figs. 4 and 5 the P.D. is so much like that of collodion particles free from albumin that the suspicion is warranted that the film of albumin had been partly or entirely destroyed by the alkalies.

lowering the surface tension of the water; but he also found that these proteins (or, perhaps, more correctly certain proteins) undergo an irreversible coagulation in this case. Applied to crystalline egg

[8] Ramsden, W., *Z. physik. Chem.*, 1904, xlvii, 336.

albumin it would mean that genuine egg albumin is denatured when it forms a film.

Herzfeld and Klinger,[9] as well as Wiechowski,[10] found that mechanical grinding of a dry powder of soluble blood albumin renders the albumin insoluble.

The mechanism of denaturation is unknown; should it be possible that the albumin molecule of the film is oriented in such a way as to render ineffective the action of the groups with a high affinity for water? The observations of Langmuir leave no doubt that the molecules of surface films are definitely oriented.

Whatever the explanation may be, the fact remains that the influence of electrolytes on the cataphoretic P.D. is practically the same for collodion particles coated with gelatin or with genuine crystalline egg albumin; while the influence of salts on the stability of suspensions of the two types of particles is entirely different. This difference finds its explanation in the fact that the forces determining the stability of suspensions of gelatin-coated particles in water are the chemical forces acting in true solubility; while the forces determining the stability of suspensions of albumin-coated collodion particles are essentially the electrostatic forces of the double electrical layer surrounding the particle.

V.

*The Stability of Suspensions of Particles of Denatured Egg Albumin.*

The conditions for the stability of suspensions of particles of denatured egg albumin are practically identical with those for the stability of suspensions of particles of collodion coated with genuine egg albumin. This supports the idea that genuine egg albumin, when forming a film on collodion, undergoes a change whereby its affinity for water no longer seems to exist.

Careful experiments on the flocculation of denatured egg albumin have been made before, especially by Chick and Martin,[11] but the cataphoretic P.D. of the particles was not measured, and we are here

[9] Herzfeld, E., and Klinger, R., *Biochem. Z.*, 1917, lxxviii, 349.
[10] Wiechowski, W., *Biochem. Z.*, 1917, lxxxi, 278.
[11] Chick, H., and Martin, C. J., *J. Physiol.*, 1912–13, xlv, 261.

concerned with the relation between that P.D. and the stability of suspensions.

Suspensions of denatured egg albumin were prepared in the following way. A 1 per cent solution of isoelectric crystalline egg albumin (pH 4.8) was heated to 90°C. and the coagulated mass was allowed to settle. It was then ground up in a mortar with a small amount of water to a milky suspension. 1 drop of a sufficiently concentrated stock suspension was put into 10 cc. of a solution of different salts at different pH, and the solution was allowed to stand over night at room temperature.

In order to bring the cataphoretic P.D. of the particles of (boiled) denatured egg albumin to zero, the surrounding solution had to have a pH of about 5.0. Flocculation occurred in all concentrations of NaCl, $Na_2SO_4$, and $CaCl_2$. Only in certain concentrations of $Na_4Fe(CN)_6$ and $LaCl_3$ was the suspension stable and these concentrations were in the case of $Na_4Fe(CN)_6$ between M/65,000 and M/16. Inside these concentrations the cataphoretic P.D. was above 10 millivolts. The suspension of particles of denatured egg albumin was stable in concentrations of $LaCl_3$ between M/2,000 and M/16, and in this case the P.D. was also above the critical level of about 12 millivolts.

At pH 4.0 the cataphoretic P.D. of the particles was about 20 millivolts without salt, and since this is above the critical value the suspension was stable. The addition of a trace of $Na_4Fe(CN)_6$, M/8,000 or less, brought the P.D. to about zero and flocculation occurred. The addition of more $Na_4Fe(CN)_6$ reversed the sign of charge and the P.D. increased. At M/2,048 the P.D. was about 12 millivolts and the suspension was stable. The other salts depressed the P.D. below the critical value in the following concentrations, NaCl M/16, $CaCl_2$ M/32, $LaCl_3$ below M/16, and $Na_2SO_4$ M/1,024; and in these and higher concentrations caused flocculation.

From these and other experiments no doubt was left that the concentration where flocculation occurs can be predicted from the cataphoretic P.D. since the suspension is stable only when the P.D. is above 10 to 12 millivolts. The maximal concentration where the suspension was stable and the minimal concentration where flocculation occurred are given in Table V.

The figures in Table V show that the suspension of particles of denatured crystalline egg albumin no longer remains stable when the P.D. falls below about 9 millivolts, while it is always stable when it is above 10 to 12 millivolts. It must also be remembered that the

TABLE V.

*Minimal Concentrations and Cataphoretic P.D. at Which Suspensions of Denatured Crystalline Egg Albumin Were Stable or Flocculated.*

| pH | | Stable. | | Complete precipitation. | |
|---|---|---|---|---|---|
| | | Concentration. | P.D. in millivolts. | Concentration. | P.D. in millivolts. |
| 11.0 | $CaCl_2$ | M/256 | 10 | M/16 | 6 |
| | NaCl | M/4 to 0. | 24 to 9. | | |
| | $Na_2SO_4$ | M/4 to 0. | 31 to 10. | 1M | <10 |
| 5.8 | NaCl | M/256 | 8 | M/128 | 7 |
| | $CaCl_2$ | M/4,096 | 8 | M/2,048 | 7 |
| | $LaCl_3$ | M/2,048 | 13 | M/32,000 | 7 |
| | $Na_2SO_4$ | M/4,096 | 12 | M/512 | 9 |
| | $Na_4Fe(CN)_6$ | | | M/4 | <9 |
| 5.0 | NaCl | | | 0 to M/4. | <3 |
| | $CaCl_2$ | | | 0 to M/8. | <3 |
| | $Na_2SO_4$ | | | 0 to M/2. | <3 |
| | $LaCl_3$ | M/2,048 to M/16. | 13 to 10. | M/32,000 to 0. | 8 to 6. |
| | $Na_4Fe(CN)_6$ | M/65,000 to M/16. | 19 to 10.5. | 0 | 5 |
| 4.0 | NaCl | M/32 | 9 | M/16 | 6 |
| | $CaCl_2$ | M/128 | 13 | M/32 | 8 |
| | $LaCl_3$ | M/65,000 to M/16. | 22 to 9. | | |
| | $Na_2SO_4$ | M/16,000 | 14 | M/1,024 | 8 |
| | $Na_4Fe(CN)_6$ | M/4,096 to M/2,048. | 12 to 8. | M/8,192 | 3 |
| 3.0 | NaCl | M/16 | 10.5 | M/4 | |
| | $CaCl_2$ | M/16 | 11 | M/4 | |
| | $LaCl_3$ | M/16 | 9.5 | M/4 | |
| | $Na_2SO_4$ | M/256 | 9.5 | M/128 | 8 |

measurements of the cataphoretic P.D. are accurate only within ± 2 millivolts.

It follows from this that the stability of suspensions of denatured crystalline egg albumin depends on the P.D. surrounding each particle.

There is, however, one statement to be added. At pH 4.0, 5.8, and 5.0, there occurs a suspension in concentrations of $CaCl_2$ and NaCl of M/2 or above. Since at these high concentrations the P.D. is very low in water, we must conclude that the salt depresses the cohesive forces between the particles, or increases the forces of attraction between water and albumin, so that the particles cannot coalesce even if the P.D. around each particle is zero. Northrop and De Kruif observed a similar phenomenon in their experiments on the influence of salts on bacterial suspensions and they proved by measurements that the cohesive forces between bacteria may be sufficiently diminished by high concentrations of salt solutions, so that no agglutination occurs between the bacteria even if the P.D. between the bacteria is low or zero.[12]

VI.

*Influence of Salts on the Heat Coagulation of Denatured Egg Albumin.*

When crystalline egg albumin is heated, it undergoes a change whereby it becomes insoluble. Its molecules upon colliding will adhere to each other and form aggregates and these aggregates may further coalesce upon colliding, provided the cataphoretic P.D. is below that of the critical value for coalescence, which was shown to be about 9 millivolts in the preceding paragraph. When the cataphoretic P.D. is above this critical value, no such coalescence will occur and the suspension will be stable. But the average size of the particles will be the smaller the higher the P.D. because the probability of the particles approaching each other with sufficient kinetic energy to break through the barrier of electrostatic repulsion becomes the smaller the higher the cataphoretic P.D. This will show itself in the appearance of the solution. When the P.D. is very high, the solution must remain clear as water, because there may be aggregates of, at the utmost, a few molecules, but no coalescence of such aggregates can occur. When the P.D. is a little less, a small percentage of particles may possess the kinetic energy to break through the barrier of electrostatic repulsion and some coalescence of aggregates may occur. Such suspensions may appear slightly bluish. Upon further dim-

[12] Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921–22, iv, 639, 655.

inution of the P.D. the relative percentage of coalescence will increase, the suspension will appear gray, finally milky, and when the P.D. falls below the critical value, coalescence will be so general that the majority of the colliding aggregates will coalesce and flocculation will occur, since the rate of settling of a suspension depends on the relative size of the particles.

7 cc. of water of pH 4.8 (this pH being the isoelectric point of crystalline egg albumin) were added to 2 cc. of 1 per cent solution of isoelectric crystalline egg albumin (of course, also of pH 4.8) and then 1 cc. of a salt solution containing different salts of different concentration, but always of pH 4.8, was added. The test-tubes containing the 10 cc. of the mixtures were put into boiling water until the liquid in the test-tubes reached a temperature of 90°C. and then the test-tubes were taken out of the water bath and allowed to cool to room temperature. Table VI gives the appearance of the various mixtures after standing over night.

Without salt the cataphoretic charge of the aggregate is zero and flocculation occurs. When either NaCl, or $Na_2SO_4$, or $CaCl_2$ is in the solution, flocculation will always occur, since these salts raise the P.D. little or not at all. When, however, $Na_4Fe(CN)_6$ or $LaCl_3$ is added, not only is there a stable suspension, but the suspension becomes almost as clear as water at that concentration of these salts at which the P.D. of denatured egg albumin is high. Thus the suspension of denatured egg albumin remains clear though slightly bluish in appearance in concentrations of $LaCl_3$ between M/2,800 and M/40 where the cataphoretic P.D. is 15 millivolts or more. At M/25 and at M/10,000 the suspension is still stable but opaque; *i.e.*, the particles are larger but not large enough to settle rapidly. At these concentrations of $LaCl_3$ the P.D. was about 10 millivolts, *i.e.* it was just above the critical value of 9 millivolts. In concentrations of M/20,000 or less $LaCl_3$ flocculation occurred, since the P.D. was below the critical value of 9 millivolts.

Acid acts in the same way. When traces of acid are added to isoelectric albumin, heat coagulation is prevented but the appearance of the solution of egg albumin after heating depends on the P.D. of the particles in the way described. 10 cc. of an aqueous 0.2 per cent solution of almost isoelectric crystalline egg albumin and containing

TABLE VI.

*Influence of Different Salts on Heat Coagulation of Crystalline Egg Albumin in Aqueous Solution at pH of Isoelectric Point.*

| Total concentration of salt in 10 cc. of 0.2 per cent albumin | 10M/80 | 8M/80 | 6M/80 | 4M/80 | 3M/80 | 2M/80 | M/80 | M/160 | M/320 | M/640 | M/1,280 | M/2,560 | M/5,120 | M/10,240 | M/20,480 | M/40,960 | M/81,920 | 0 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $LaCl_3$ | Coagulated. | | | | Opalescent. | Bluish clear. Clearest. | | | | | | | Opalescent and turbid. | Milky. | Coagulated. | | | |
| $Na_4Fe(CN)_6$ | | Very opaque. | Slight opalescence. | Clear. | | | | | | Increasing opalescence. → | | | Milky. | | | | Coagulated. | |
| $BaCl_2$ | Coagulated. | | | | | | | | | | | | | | | | | |
| $CaCl_2$ | Coagulated. | | | | | | | | | | | | | | | | | |
| $Na_2SO_4$ | Coagulated. | | | | | | | | | | | | | | | | | |
| NaCl | Coagulated. | | | | | | | | | | | | | | | | | |

TABLE VII.

*Influence of HCl on Heat Coagulation of Crystalline Egg Albumin in Aqueous Solution. 10 cc. of 0.2 Per Cent Albumin (Nearly Isoelectric), Containing Various Concentrations of 0.1 N HCl, Heated to 90°C.*

| cc. 0.1 N HCl in 10 cc. of 0.2 per cent albumin | 0 | 0.01 | 0.02 | 0.03 | 0.04 | 0.05 | 0.1 | 0.2 | 0.4 | 0.8 | 1.6 | 3.2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Appearance of solution | Coagulated. | | Very opaque. | Very opalescent. | Clear but slightly opalescent. | Very clear, like water. | | | | | | |

varying amounts of 0.1 N HCl were put into test-tubes, and these test-tubes were put into boiling water until the temperature of the albumin solution rose to 90°C. Then the test-tubes were allowed to cool to room temperature and the appearance of the solution was noticed. Table VII gives the result.

TABLE VIII.

*Heat Coagulation of Crystalline Egg Albumin.*

| pH | | No coagulation. | | Coagulation. | |
|---|---|---|---|---|---|
| | | Concentration. | P.D. in millivolts. | Concentration. | P.D. in millivolts. |
| 11.0 | $NaCl$ | 0 to M/2. | 24 to 9. | | |
| | $CaCl_2$ | 0 to M/512. | 31 to 12.5. | M/128 to 2 M. | 8.5 and less. |
| | $Na_2SO_4$ | 0 to M/8. | 31 to 12. | 1 M | |
| | $Na_4Fe(CN)_6$ | 0 to M/8. | 29 to 9.5. | M/2 | |
| 5.8 | $NaCl$ | | | M/16 and above. | 5 and less. |
| | $CaCl_2$ | | | M/512 to 1 M. | 5 and less. |
| | $Na_2SO_4$ | | | M/16 to M/2. | 7 and less. |
| | $Na_4Fe(CN)_6$ | | | M/4 | |
| 4.0 | $NaCl$ | | | M/64 to 2 M. | 11 and less. |
| | $CaCl_2$ | | | M/128 to 2 M. | 13 and less. |
| | $LaCl_3$ | | | M/4 | |
| | $Na_2SO_4$ | | | M/1,024 to 1 M. | 8 and less. |
| 3.0 | $NaCl$ | M/32 | 13.5 | M/4 | |
| | $CaCl_2$ | M/32 | 14 | M/8 to 2 M. | 8 and less. |
| | $LaCl_3$ | M/32 | 13 | M/4 | |
| | $Na_2SO_4$ | M/512 | 10 | M/128 | 8 and less. |
| 4.8 | $NaCl$ | | | At all concentrations. | About 3 or less. |
| | $CaCl$ | | | | |
| | $Na_2SO_4$ | | | | |
| | $LaCl_3$ | M/2,500 to M/26. | 13 to 11. | | |
| | $Na_4Fe(CN)_6$ | M/2,500 to M/20. | 30 to 14. | | |

When the 10 cc. contained 0.01 cc. of 0.1 N HCl the protein remained practically isoelectric (pH 4.8), the P.D. remained below that of the critical point, and hence flocculation occurred upon heating. When 0.02 cc. of 0.1 N HCl was added, coagulation no longer occurred,

but enough particles could coalesce because the P.D. was not very high. Only when 0.05 cc. or more acid was added did the cataphoretic P.D. become high enough to keep the solution clear on boiling. When the concentration of acid was sufficiently increased so as to bring the cataphoretic P.D. down again below the critical value, flocculation occurred again.[13]

Table VIII compares the maximal concentrations of salts at which solutions of genuine crystalline egg albumin no longer flocculate upon heating to 90°C. and the minimum concentrations required for flocculation. The table also gives the cataphoretic P.D. of denatured particles of white of egg at these concentrations.

## VII.

### *Proteins as Protective Colloids.*

In his experiments on anomalous osmosis the writer showed that when collodion membranes are filled with a 1 per cent solution of a protein, such as gelatin, crystalline egg albumin, casein, or oxyhemoglobin, there is formed over night inside the membrane a durable film of solid protein which cannot be washed away even if the interior is rinsed out as often as ten or twenty times with warm water.[4] This film betrays itself by its color in the case of oxyhemoglobin. The forces which make the film adhere to the collodion must be very strong, but they do not depend upon the ionization of the protein, since the films are formed no matter whether the protein is at the isoelectric point, or whether it is on the alkaline or on the acid side of the isoelectric point. The forces which cause the film formation must be those forces of secondary valency responsible for phenomena of adhesion and cohesion in general.

This film formation is responsible for the so called protective action of certain colloids. Zsigmondy and his collaborators showed that

[13] Some of these results had been discussed in a preceding paper (*J. Gen. Physiol.*, 1921–22, iv, 759) on the assumption that the double electrical layer is determined by the membrane equilibrium. Since it seems that membrane potentials and cataphoretic potentials are not identical and since the stability of suspensions seems to depend on cataphoretic potentials, it seemed necessary to interpret these phenomena on the basis of cataphoretic potentials.

suspensions of colloidal gold, which were precipitated by low concentrations of salts, were protected against such precipitation when the gold particles were suspended in a gelatin solution; and to make the work quantitative he introduced the term gold number, defining it as that number of milligrams of protective substance which is just sufficient to prevent a definite degree of agglutination of the gold particles caused by the addition of 1 cc. of 10 per cent NaCl to 10 cc. of suspension of gold particles.[14]

Gelatin was found to be especially active as a protective colloid, while egg albumin—which Zsigmondy did not purify by crystallization—had little protective action. The experiments reported in this paper give an explanation why gelatin is a good protective colloid and why crystalline egg albumin is not. Suspensions of collodion particles not treated with protein are precipitated by low concentrations of salt because the particles are kept in suspension only by virtue of their double electrical layers, the P.D. of which is brought below the critical value by comparatively low concentrations of salts. When collodion particles are put into a solution of gelatin, a gelatin film is formed at the surface, the molecules of which retain the high affinity of gelatin for water, and this affinity is not destroyed by even very high concentrations of salts. Consequently the stability of the suspension of gelatin-coated collodion particles no longer depends on the double electrical layer which determined the stability of the collodion particles before they were coated with protein, and which is reduced below the critical value by relatively low concentrations of salts.

When genuine crystalline egg albumin forms a film around a collodion particle, the albumin loses its high affinity for water and behaves like denatured egg albumin, inasmuch as its affinity for water molecules is considerably diminished. Collodion particles coated with egg albumin depend therefore chiefly on the electrical double layer surrounding each particle and hence will be precipitated by low concentrations of salts.

If we take the effects of the hydrogen ion concentration into consideration, we can, however, single out certain cases where even a

[14] Zsigmondy, R., Kolloidchemie, Leipsic, 1918, 173, 358.

film of crystalline egg albumin has some protective action on suspensions of collodion particles. Thus, a low concentration of $LaCl_3$ (about M/4,000) suffices to flocculate a suspension of collodion particles free from protein at pH 4.0 or 3.0. When, however, the particles are coated with egg albumin or casein, the concentration of $LaCl_3$ required for that purpose is much higher, about M/32 or even higher; since at the pH mentioned, the particles are positively charged and the P.D. is diminished by the Cl ion instead of by the La ion. A second protective effect is noticed at pH 4.0 or pH 4.7 in high concentrations of $CaCl_2$, M/2 or somewhat higher, when the particles are coated with casein or albumin.

Casein has generally little or no protective action, since it will be shown in a subsequent paper that the stability of casein-coated collodion particles depends on the cataphoretic P.D. which is depressed below the critical value by low concentrations of salts. In certain cases, however, salts, especially $CaCl_2$ in high concentrations, can keep the particles in suspension even if the P.D. is zero.

Experiments with edestin showed that it is of very little use as a protective colloid.

These experiments permit us to define the conditions for a general protective action of colloids, such as that by gelatin. Protective colloids must first be capable of forming durable films on the surface of the particles to be protected, and, second, the molecules constituting the film must have a higher attraction for the molecules of the solvent (*e.g.* water) than for each other; in other words, they must possess true crystalloidal solubility. Those who refuse to believe that proteins may form true solutions will find it difficult to explain the mechanism of the protective action of such colloids as gelatin.

We have no idea of the mechanism whereby a protein can act as an antigen; but it is, perhaps, worth while to point out that gelatin which forms films with a high affinity for water is no antigen, while crystalline egg albumin, casein, and edestin, which form films with practically no affinity for water, are good antigens. It is, however, quite possible that the coincidence is merely accidental.

## VIII.

## SUMMARY.

1. It is shown that the concentrations of different salts required to precipitate *suspensions* of gelatin-coated collodion particles in water are practically identical with the concentrations of the same salts required for the "salting out" of gelatin from aqueous *solutions*. Neither effect shows any relation to the electrical double layers surrounding the particles.

2. It is shown that at the isoelectric point of gelatin, *suspensions* of gelatin-coated collodion particles are not stable and it had been shown previously that gelatin is least soluble at the isoelectric point. The addition of salt increases both the solubility of gelatin in water as well as the stability of suspensions of gelatin-coated collodion particles in water, and both effects increase with the valency of one of the ions of the salt.

3. This latter effect is not due to any charges conferred on the gelatin particles by the salts, since the cataphoretic experiments show that salts like NaCl, $Na_2SO_4$, or $CaCl_2$, which at the isoelectric point of gelatin increase the solubility of gelatin as well as the stability of suspensions of gelatin-coated collodion particles, leave the particles practically uncharged in the concentrations in which the salts are efficient.

4. It follows from all these facts that the stability of suspensions of gelatin-coated particles in water depends on the solubility of gelatin in water; *e.g.*, on the chemical affinity of certain groups of the gelatin molecule for water.

5. Though crystalline egg albumin is highly soluble in water, the stability of collodion particles coated with crystalline egg albumin does not depend upon the affinity of the albumin molecule for water, but depends practically alone on the electrical double layer surrounding each particle. As soon as the P.D. of this double layer falls below 13 millivolts, the suspension is no longer stable.

6. The critical potential for the stability of suspensions of collodion particles coated with genuine egg albumin is the same as that for particles of boiled (denatured) white of egg. Since through the process of heating, egg albumin loses its solubility in water, it is inferred that

egg albumin undergoes the same change when it forms a film around a solid particle like collodion.

7. The influence of electrolytes on the stability of suspensions of collodion particles coated with casein or edestin was similar to that of collodion particles coated with egg albumin. The experiments are, however, complicated by the fact that near the isoelectric point $CaCl_2$ and even NaCl cause a suspension again at concentrations of about M/2 or 1 M, while still higher concentrations may cause a precipitation again. These latter effects have no connection with double layers, but belong probably in the category of solubility phenomena.

8. These experiments permit us to define more definitely the conditions for a general protective action of colloids. Protective colloids must be capable of forming a durable film on the surface of the suspended particles and the molecules constituting the film must have a higher attraction for the molecules of the solvent than for each other; in other words, they must possess true solubility. Only in this case can they prevent the precipitating action of low concentrations of electrolytes on particles which are kept in suspension solely by the high potentials of an electrical double layer. Thus gelatin films, in which the attraction of the molecules for water is preserved, have a general protective action, while crystalline egg albumin, casein, and edestin, which seem to lose their attraction for water when forming a film, have a protective action only under limited conditions stated in the paper.

# MEMBRANE POTENTIALS AND CATAPHORETIC POTENTIALS OF PROTEINS.

By JACQUES LOEB.

*(From the Laboratories of The Rockefeller Institute for Medical Research.)*

(Received for publication, January 15, 1923.)

## I.

Hardy observed in 1900 that particles of denatured (boiled) white of egg migrated in an electric field to the cathode in an acid solution, to the anode in alkaline solution, and did not migrate at all at a point between the two; namely, at the so called isoelectric point.[1] It was shown in previous publications[2] that the same influence of the pH on the charge of the protein exists in the case of membrane potentials, inasmuch as a protein solution enclosed in a collodion bag and submerged in water free from protein is positively charged on the acid side of the isoelectric point, negatively on the alkaline side, and not charged at all at the isoelectric point. The question arises, What is the cause of the similarity of the influence of the pH on the two types of potentials? The membrane potentials are due to a difference in the concentration of a diffusible ion, *e.g.* the H ion inside the protein solution and outside; and the membrane potentials can be calculated with a fair degree of accuracy from the ratio of the hydrogen ion concentrations inside and outside with the aid of Donnan's formula. The cataphoretic potentials, however, are determined by the potential difference between the two strata of an electrical double layer situated at the interface between particles and water, but entirely in the water. One stratum or film of this double layer, namely the one adjoining the solid particle, adheres to the solid particle and moves with it, and the charge of this film is the cause of the cataphoretic motion of the particles.

[1] Hardy, W. B., *Proc. Roy. Soc. London*, 1899–1900, lxvi, 110.

[2] Loeb, J., *J. Gen. Physiol.*, 1920–21, iii, 667. Proteins and the theory of colloidal behavior, New York and London, 1922.

According to the theory of these double layers originally developed by Helmholtz and modified in an essential point by Perrin, the potential difference of this electrical double layer can be calculated from measurements of the velocity of migration of such particles in an electric field with the aid of the following formula

$$v = \frac{\epsilon \cdot E \cdot K}{4 \pi \eta}$$

where $v$ is the velocity of migration of the particle in centimeter per second, $\epsilon$ the potential difference between the two strata of the double layer around the solid particle, $E$ the potential gradient in E. S. U. per centimeter of the galvanic field, $K$ the dielectric constant of the water or the solution, and $\eta$ the viscosity of the water. It may be said that this formula must be nearly correct for the reason that flocculation of suspensions of a given substance always occurs at the same calculated cataphoretic P. D., which would be impossible if the Helmholtz-Perrin formula were not, at least approximately, correct. We shall return to this point later.

We are not so well informed as to the origin of the P.D. of this double layer, but we may assume with a good degree of probability that it is due to the fact that the two oppositely charged ions of an electrolyte are not contained in the same concentration in the two strata of the double layer, and that forces inherent in the water drive an excess of one type of ions—generally the OH or some other negative ion—into the outermost surface of the water, *i.e.* into that film or stratum of the interface which adheres to and moves with the solid particle. Since this film determines the cataphoretic sign of charge of the particle, we notice that, very frequently, suspended particles are negatively charged in water, while the bulk of water, having a corresponding excess of positive ions, is positively charged.

It is obvious, therefore, that, as a rule, the cataphoretic potential has an entirely different origin from the membrane potentials, and this makes it more difficult to account for the fact that the sign of charge of membrane potentials and of cataphoretic potentials of protein particles varies in the same sense with the change in the hydrogen ion concentration.

It was, therefore, important to find out how far the agreement between the two potentials actually goes. For this purpose the measurements of the influence of salts on the cataphoretic potentials of solid protein particles, described in a preceding paper,[3] were used.

In these experiments the following data are of importance for us. At the isoelectric point the charge of the particles is zero, if no salt is present. The addition of NaCl, $Na_2SO_4$, and $CaCl_2$ had very little effect on the cataphoretic P.D. except that $CaCl_2$ made the particles slightly positive and $Na_2SO_4$ slightly negative. While these effects were very slight, they seemed to exist in the case of all proteins.

The effects of $LaCl_3$ and of $Na_4Fe(CN)_6$ on the P.D. of the isoelectric casein particles were much greater. $Na_4Fe(CN)_6$ made the particles strongly negative, while $LaCl_3$ made them strongly positive.

At pH 4.0, the protein particles, such as gelatin, casein, and albumin are positively charged without salts, the cataphoretic P.D. being in the neighborhood of 15 millivolts. $LaCl_3$, $CaCl_2$, and NaCl depressed the P.D., and the more the higher the concentration of the salt, and $Na_2SO_4$ depressed the cataphoretic P.D. still more rapidly. All these effects of salts on the cataphoretic P.D. are similar to the effects of these salts on the membrane potentials at the same pH. What is, however, different is the effect of $Na_4Fe(CN)_6$, which reverses the sign of the cataphoretic charge of the particles in as low a concentration as M/65,000. We shall see later that such a reversal of the sign of charge of proteins by $Na_4Fe(CN)_6$ occurs only in the case of the cataphoretic, but not in the case of membrane potentials of proteins at a pH of 4.0.

At pH 3.0, the above mentioned protein particles are positively charged without salts, the P.D. being about 20 millivolts. At this pH the influence of $Na_4Fe(CN)_6$ can no longer be investigated on account of the chemical changes in the salt. All the four salts, NaCl, $Na_2SO_4$, $CaCl_2$, and $LaCl_3$, depressed the cataphoretic P.D., and $Na_2SO_4$ more rapidly than the three chlorides. This effect of these salts on the cataphoretic P.D. is similar to their effect on the membrane potentials at pH 3.0.

[3] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 395.

Without salts the protein particles were negatively charged at pH 5.8, the P.D. being about 12 millivolts. $LaCl_3$ reverses the sign of charge in as low a concentration as M/30,000, while NaCl, $CaCl_2$, and $Na_2SO_4$ cause no such reversal. $Na_4Fe(CN)_6$ causes an enormous increase in the negative charge of the particles, while $Na_2SO_4$ causes a slight increase. $CaCl_2$ causes no increase in the cataphoretic P.D. at pH 5.8. Neither the reversal of the sign of charge by $LaCl_3$ nor the increase of the charge by $Na_4Fe(CN)_6$ at pH 5.8 was observed in the case of membrane potentials.

These experiments, then, prove the existence of definite differences between membrane potentials and cataphoretic potentials.

II.

To leave no doubt that these differences are real, experiments were made on the effect of $Na_4Fe(CN)_6$ and $LaCl_3$ on the membrane potentials of 3 per cent and 1 per cent solutions of crystalline egg albumin at pH 4.0 and 5.8 respectively. The membrane potentials were measured after 18 hours, in the way described in a previous publication.

Fig. 1 gives a comparison of the effects of different concentrations of $Na_4Fe(CN)_6$ on the cataphoretic potentials of albumin-coated collodion particles (upper curve) and on the membrane potentials of a 3 per cent and 1 per cent solution of crystalline egg albumin at a pH of 4.0. The ordinates are the P.D. in millivolts, while the abscissæ are the concentrations of $Na_4Fe(CN)_6$ used. When the protein is positively charged, the P.D. is below the zero line; and when the protein is negatively charged, the P.D. is above the zero line.

Without salt the protein at pH 4.0 is positively charged in both membrane and cataphoretic potentials, but the membrane potential is higher than the cataphoretic potential. While a concentration of M/200,000 $Na_4Fe(CN)_6$ suffices to reverse the sign of charge of the protein in the case of cataphoretic potentials, no such reversal occurs in the case of the membrane potentials of the 3 per cent albumin solution. In this latter case the salt depresses the P.D. in accordance with Donnan's theory and at a concentration of M/1,024 the P.D. is zero and stays so even if the concentration of the salt is as high as M/64 or above.

A slight reversal seems to occur in the case of the membrane potentials of a 1 per cent solution of albumin at a concentration of M/2,048; but this reversal is in reality due to a change of the pH in the protein solution caused by the $Na_4Fe(CN)_6$ on standing. Measurements of

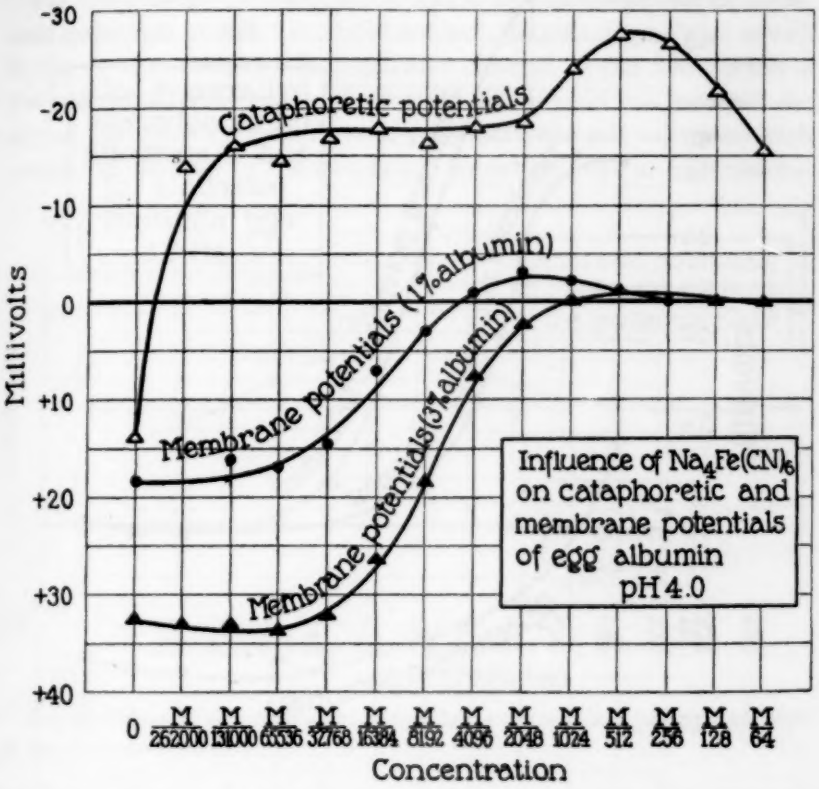


FIG. 1. Comparison of the influence of $Na_4Fe(CN)_6$ on membrane potentials of 1 per cent and 3 per cent solutions of crystalline egg albumin and the cataphoretic potentials of collodion particles coated with crystalline egg albumin at pH 4.0. While low concentrations of the salt reverse the sign of charge of the cataphoretic potentials, no reversal occurs in the case of membrane potentials.

the pH of the protein solution show that it rises in 18 hours beyond that of the isoelectric point and this causes the reversal of the sign of charge at M/4,096 or M/2,048 $Na_4Fe(CN)_6$. When the concentration of the salt becomes higher the depressing effect of the salt brings

the membrane potentials again to zero. This reversal of the membrane potentials, due to a change in the pH, did not occur in the 3

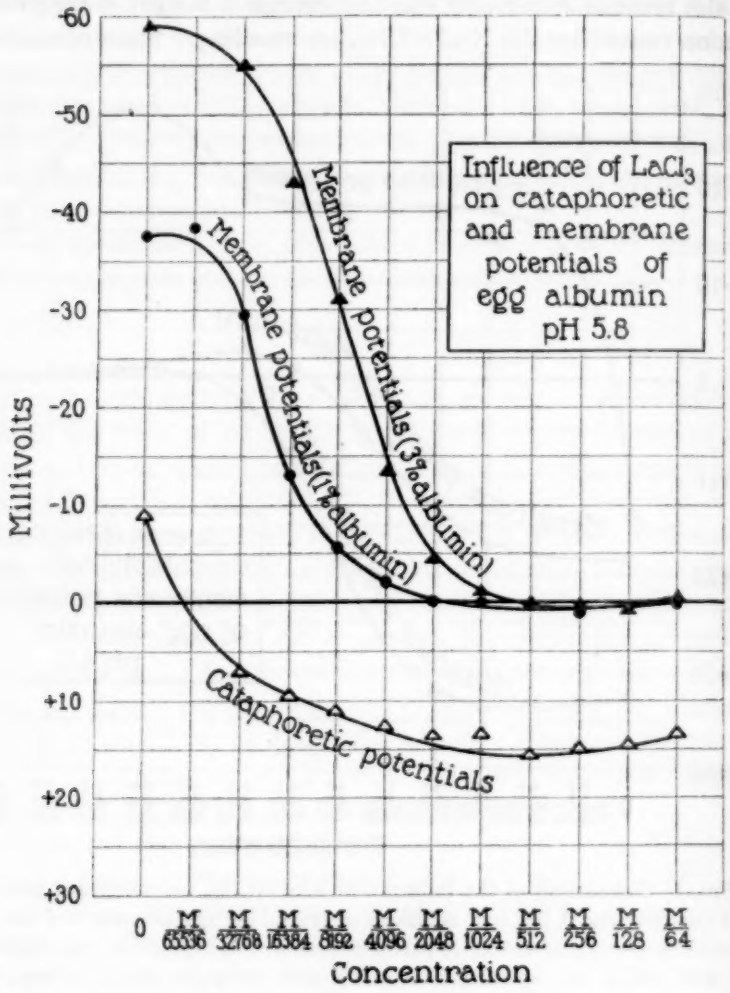


FIG. 2. Comparison of influence of $LaCl_3$ on membrane potentials and cataphoretic potentials of albumin at pH 5.8.

per cent protein solution, possibly because the protein acts as a buffer against the pH changes and this buffer action is the greater the higher the concentration of the protein. In the measurements of the cataph-

oretic potentials no such pH changes occurred, since the measurements of the cataphoretic potentials were made after 20 minutes instead of after 18 hours. In 20 minutes the pH undergoes no material change.

Fig. 2 compares the influence of $LaCl_3$ on membrane and cataphoretic potentials at pH 5.8. Without salt the protein particles as well as the protein solution are negatively charged at pH 5.8. While a low concentration of $LaCl_3$, about M/32,000 or even less, reverses the sign of charge of the cataphoretic potentials, the salt causes no such reversal in the case of the membrane potentials even in high concen-

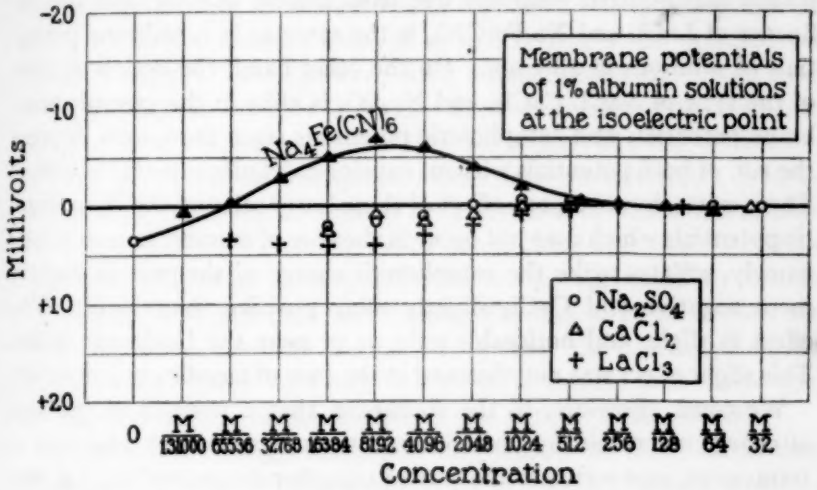


FIG. 3. Influence of various salts on membrane potentials of albumin solutions at the isoelectric point.

trations. $LaCl_3$ can only bring the membrane potentials of a protein solution at pH 5.8 to zero but cannot reverse their sign of charge.

Near the isoelectric point, low concentrations of $Na_4Fe(CN)_6$ produce a considerable negative cataphoretic charge, and low concentrations of $LaCl_3$, produce a considerable positive cataphoretic charge of the albumin particle. Fig. 3 shows that they produce no such charge in the membrane potentials of albumin solution at the isoelectric point except that caused by a change in the hydrogen ion concentration of the protein solution by the salt. $Na_4Fe(CN)_6$ brings the solution of crystalline egg albumin in 18 hours to a pH slightly above 4.7.

Previous experiments on the influence of $Na_4Fe(CN)_6$ or $LaCl_3$ on the membrane potentials of gelatin solutions at the isoelectric point had given results similar to the new experiments on egg albumin, and the writer also noticed a tendency of the solution to change its pH.[4] The writer was not certain at that time that the slight effect of $Na_4Fe(CN)_6$ and $LaCl_3$ on the membrane potentials of isoelectric gelatin was due exclusively to a change of the pH occurring gradually on standing. The new experiments make it more probable that this must have been the case.

Experiments on the membrane potentials between solid gels of gelatin and aqueous solutions free from gelatin showed that the influence of $LaCl_3$ and $Na_4Fe(CN)_6$ is the same as in membrane potentials of solutions of albumin. On the other hand, the action of salts of the type of NaCl, $CaCl_2$, and $Na_2SO_4$ is alike in the case of membrane potentials and cataphoretic potentials since these salts depress the P.D. of both potentials without causing a definite reversal of either. There exists, however, one effect of these latter salts on the cataphoretic potentials which does not occur in the case of membrane potentials; namely, sulfates make the cataphoretic charge of the protein slightly more negative and $CaCl_2$ slightly more positive than NaCl. The effect is slight and noticeable only at or near the isoelectric point. This slight effect was not observed in the case of membrane potentials.

We come, therefore, to the conclusion that a reversal of the sign of charge of protein by low concentrations of salts with trivalent or tetravalent ions occurs in the case of cataphoretic potentials, but not, or practically not, in the case of membrane potentials. The fact that such a reversal is brought about in the cataphoretic potentials by low concentrations of $LaCl_3$ and $Na_4Fe(CN)_6$ was corroborated by experiments on two types of phenomena which depend on cataphoretic potentials; namely, on the stability of protein particles and on electrical osmosis through protein films.

If we assume the validity of the Helmholtz-Perrin theory of cataphoretic migration, the sign of cataphoretic migration is determined by that film of water which adheres to and moves with the protein particle. This film is usually negatively charged, probably because it has an excess of negative ions which are forced into the film by

[4] Loeb, J., *J. Gen. Physiol.*, 1921–22, iv, 741.

forces inherent in the water—presumably surface tension forces. If the two ions of an electrolyte lower the surface tension of water to a different extent, that ion must be driven in excess into the outermost stratum of the double layer (*i.e.* into the stratum which adheres to and moves with the particle) which has the greater depressing effect on the surface energy. We may conceive that the molecules of water are oriented by such forces at the surface of the water, the oxygen atom forming, as a rule, the outermost, the hydrogen the deeper stratum of the surface.

While thus negative ions are generally forced in excess into the outermost stratum at the surface of the water, the positive ions are in excess in the stratum beneath or in the bulk of the solution. It seems, however, that the force with which cations are driven away from the surface deeper into the water decreases with increasing valency of the cation, so that in the case of salts like $LaCl_3$ the trivalent La ion is driven with greater force into the outermost stratum of the water than the Cl ions, as a consequence of which this stratum becomes positive. That the force determining this ionic stratification may be surface tension seems to be also supported by the fact (observed by Freundlich and Gyemant[5]) that organic cations like basic dyes have also the tendency to reverse the generally negative cataphoretic charge of "oily" particles in water, such basic dyes having a tendency to accumulate in the surface of the water.

These facts and suggestions probably explain the fact that particles of gelatin chloride of pH 4.0, which are positively charged, assume a negative cataphoretic charge in a weak solution of $Na_4Fe(CN)_6$; while particles of Na gelatinate of pH 5.8, which are negatively charged, assume a strong positive charge in a solution of $LaCl_3$. We may also understand on this basis why $Na_2SO_4$ has a tendency to make the cataphoretic charge of the protein particles near the isoelectric point slightly more negative and why $CaCl_2$ makes the particles a little more positive than does NaCl.

All these facts agree with the idea that the cataphoretic migration is, indeed, determined by the P.D. of an electrical double layer situated entirely in the water and determined at least partly by forces inherent in the water.

[5] Freundlich, H., and Gyemant, A., *Z. physik. Chem.*, 1922, c, 182.

III.

But this leaves the equally striking fact unexplained that changes in the hydrogen ion concentration affect the cataphoretic P.D. of protein particles similarly as they affect the membrane potentials of protein solutions. The influence of acids and alkalies on the cataphoretic P.D. of gelatin-coated particles of collodion had been shown in a preceding article.[3] At the isoelectric point the cataphoretic P.D. of gelatin-coated collodion particles was zero, but both acid and alkali increased the P.D., the particles being negatively charged in alkali and positively charged in acid, just as in the case of the membrane potentials. When the anion of the acid or the cation of the alkali was monovalent, the cataphoretic P.D. was greater than when the respective ions were bivalent. This agrees with the valency effect in the case of membrane potentials.

When the concentration of acid or alkali exceeded a certain limit, the further increase in concentration diminished the cataphoretic P.D. again, and this was also the case with the membrane potentials. It would have been of importance to find out whether the agreement was quantitative, but this was impossible, since we do not know the concentration of protein in the solid particles. The membrane potential of a 1 per cent solution of protein was for the same pH always greater than the cataphoretic P.D. of solid particles of the same protein.

The question arises, What causes this qualitative agreement between the two potentials in regard to the pH effect? We have a mathematical theory of the effect of acid and alkali on the membrane potentials but, unfortunately, not on the cataphoretic potentials, so that we cannot offer more than a provisional suggestion.

On the basis of Helmholtz's theory of double layers the motion of protein particles to the cathode must be due to an excess of H (or other positive) ions in that film of water which adheres to and moves with the protein particles; and the motion of the solid protein particles to the anode must be due to an excess of OH or other anions in the same film of water. This film of water must, therefore, have an excess of cations when the pH of the protein particle is on the acid side of the isoelectric point, and an excess of anions when the pH is on the

alkaline side of the isoelectric point of the protein; and this excess of cations and anions respectively in the layer of water adhering to the protein particle (and not the charges of the protein particle itself) must, on the basis of Helmholtz's theory of double electrical layers, be responsible for the sign of the cataphoretic migration of the particles.

The difficulty which confronts us is the uncertainty concerning the exact location of the P.D. determining the membrane potentials of protein particles. J. A. Wilson[6] assumes that the membrane potential between a solid protein particle and the solution is located at the boundary between a stratum of liquid adhering to the surface of the particle and the bulk of the solution. This assumption is based on the idea that the concentration of diffusible ions, *e.g.* H and Cl, at the surface of a particle of gelatin chloride will be the same as in the interior of the gel. On that assumption the P.D. of the membrane potential must be located between this surface stratum of liquid and the bulk of solution.

Now if this surface stratum of liquid is identical with the stratum of liquid which adheres to the particle in its cataphoretic motion, it is obvious why a solid particle of protein chloride must move to the cathode and a solid particle of Na proteinate must move to the anode. The ultimate cataphoretic P.D. would then be the resultant between the membrane potentials and the P.D. determined by the (surface tension?) forces inherent in the water.

On this assumption it is difficult to understand why there should ever be a difference between the membrane potentials and the cataphoretic potentials, and yet our experiments have shown such a difference to exist. We can only state at present that the cataphoretic P.D. of the protein particles is influenced both by the (surface tension) forces inherent in the water and the membrane potentials.

## IV.

Throughout this paper we have assumed the correctness of the Helmholtz-Perrin formula for the calculation of the cataphoretic P.D. which had been used in our experiments. The question might be raised whether the discrepancies between the influence of salts on

[6] Wilson, J. A., *J. Am. Chem. Soc.*, 1916, xxxviii, 1982.

the cataphoretic potentials and the membrane potentials might not be due to the fact that the Helmholtz-Perrin formula for the calculation of the cataphoretic P.D. is not correct, and that if this formula is corrected the discrepancies might disappear. It should be pointed out that there exists a group of facts which offers a strong support for the assumption that the Helmholtz-Perrin formula for the calculation of the cataphoretic P.D. cannot be far from correct; namely, the existence of a critical P.D. for the flocculation of suspensions. Regardless of the nature of the salt used, flocculation of suspensions of collodion particles occurs always when the P.D. falls below a certain critical value.[7] Powis[8] had observed the same fact in his experiments on emulsions, and Northrop and De Kruif[9] in suspensions of bacteria. Moreover, the numerous observations of Burton, at least, do not contradict the fact. Furthermore, in the next paper we shall see that a critical potential for flocculation exists also in the case of suspended particles of proteins. This seems, therefore, to justify our assumption that the figures for the cataphoretic potentials given in this paper are essentially correct. The critical P. D. is apparently different for different substances.

V.

Freundlich and Rona[10] have noticed a difference between Haber's phase boundary potentials at the boundary of glass and water and the cataphoretic potentials of water against glass. The phase boundary potential depends in this case only on the hydrogen ion concentration of the solution, while other ions, except H and OH, have no direct influence on this potential, as had already been shown by Haber and Klemensiewicz.[11] The "electrokinetic potential" at the boundary of glass and water measured cataphoretically by Freundlich and Rona showed, however, a striking influence of other ions besides hydrogen and hydroxyl ions, and showed especially the

[7] Loeb, J., *J. Gen. Physiol.*, 1922–23, v, 109.

[8] Powis, F., *Z. physik. Chem.*, 1914, lxxxix, 186.

[9] Northrop, J. H., and De Kruif, P. H., *J. Gen. Physiol.*, 1921–22, iv, 639, 655.

[10] Freundlich, H., and Rona, P., *Sitzungsber. Preuss. Akad. Wissensch.*, 1920, xx, 397.

[11] Haber, F., and Klemensiewicz, Z., *Z. physik. Chem.*, 1909, lxvii, 385.

valency effect so characteristic of all cataphoretic potentials. Freundlich and Rona assume that the difference between the two kinds of potential is as follows. The phase boundary potential is the potential difference between the interior of the solid phase and the interior of the liquid and is therefore influenced only by such ions of the liquid which can go into the solid phase, and which seem to be in the case of glass only H and OH ions. The electrokinetic potential, however, is in accordance with Helmholtz's theory, the potential difference between a film of water adhering to the solid particle and the interior of the water. This P.D. of the double electrical layer is influenced by all the ions of the liquid and the authors assume that adsorption plays the chief rôle in the electrokinetic potentials.

Freundlich and Gyemant[5] compared the thermodynamic and electrokinetic potentials between water-immiscible liquids (phenol, guaiacol, benzonitrile, and aniline) and aqueous solutions, and confirmed the conclusions arrived at by Freundlich and Rona. The thermodynamic potentials between these "oily" liquids and water had been investigated by Beutner[12] in a series of excellent experiments, and his results and conclusions in regard to the origin of these potentials were confirmed by Freundlich and Gyemant. Beutner found that the non-aqueous phase was the more positively or negatively charged the more soluble the cation or anion of a salt was respectively in the non-aqueous phase. Freundlich and Gyemant found that in the cataphoretic potentials between these four non-aqueous liquids and water the non-aqueous droplets were always negatively charged, even the basic aniline, and the sign of the cataphoretic charge of these water-immiscible droplets could be reversed by polyvalent inorganic cations and by organic cations (*e.g.* basic dyes). This influence of the cations on the cataphoretic potentials they ascribe to adsorption.

These experiments bring out the difference between thermodynamic potentials and cataphoretic potentials in the cases which Freundlich and his collaborators investigated. The ideas on adsorption can, however, not be used to explain why the membrane potentials of proteins are modified in the same way by H and OH ions as are the

[12] Beutner, R., Die Entstehung elektrischer Ströme in lebenden Geweben, Stuttgart, 1920.

cataphoretic potentials, since adsorption, according to Freundlich and Rona, influences only the cataphoretic potentials.

We may state, as a result of our experiments, that the cataphoretic migration and the cataphoretic P.D. of protein particles or of suspended particles coated with a protein are the result of two groups of forces; namely, first, forces inherent in the protein particles (these forces being linked with the membrane equilibrium between protein particles and the outside aqueous solution); and second, forces inherent entirely in the aqueous solution surrounding the protein particles.

The forces inherent in the protein particles and linked with the membrane equilibrium prevail to such an extent over the forces inherent in the water, that the sense of the cataphoretic migration of protein particles is determined by the forces resulting from the membrane equilibrium.

## VI.

## SUMMARY AND CONCLUSIONS.

1. It has been shown in preceding publications that the membrane potentials of protein solutions or gels are determined by differences in the concentration of a common ion (*e.g.* hydrogen ion) inside a protein solution or protein gel and an outside aqueous solution free from protein, and that the membrane potentials can be calculated with a good degree of accuracy from Donnan's equation for membrane equilibria.

2. On the basis of the theory of electrical double layers developed by Helmholtz, we are forced to assume that the cataphoretic potentials of protein particles are determined by a difference in the concentration of the two oppositely charged ions of the same electrolyte in the two strata of an electrical double layer surrounding the protein particle but situated entirely in the aqueous solution.

3. The membrane potentials of proteins agree with the cataphoretic potentials in that the sign of charge of the protein is negative on the alkaline side and positive on the acid side of the isoelectric point of the protein in both membrane potentials and cataphoretic potentials. The two types of potential of proteins disagree, especially in regard to the action of salts with trivalent and tetravalent ions on the sign of charge of the protein. While low concentrations of these salts

bring about a reversal of the sign of the cataphoretic potentials of protein particles (at least in the neighborhood of the isoelectric point), the same salts can bring the membrane potentials of proteins only to zero, but can bring about no or practically no reversal of the sign of charge of the protein. Where salts seem to bring about a reversal in the membrane potential of protein solutions, the reversal is probably in reality always due to a change in the pH.

4. We may state, as a result of our experiments, that the cataphoretic migration and the cataphoretic P.D. of protein particles or of suspended particles coated with a protein are the result of two groups of forces; namely, first, forces inherent in the protein particles (these forces being linked with the membrane equilibrium between protein particles and the outside aqueous solution); and second, forces inherent entirely in the aqueous solution surrounding the protein particles.

The forces inherent in the protein particles and linked with the membrane equilibrium prevail to such an extent over the forces inherent in the water, that the sense of the cataphoretic migration of protein particles is determined by the forces resulting from the membrane equilibrium.